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Research Article

Nutritional and Immunopotentiating Function of Methionine for Poultry

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Abstract

As an essential nutrient and a first limiting amino acid in several meal-based poultry diets, methionine (Met) is of rich nutritional value and physiological functions. Methionine's multiple functions include but not limited to protein synthesis, feather development, and protection from oxidative stress, methylation of DNA reactions as well as several distinct molecules.

Methionine is suggested to play an influential role in both humoral and cell-mediated immune responses. This is vital as the immune system prospers immensely from adequate nutrition, constituting of a balanced diet with supplementation of certain essential nutrients. Methionine's immunopotentiating roles include detoxification, increasing humoral immune response, stimulation of the phagocytic activity of leukocyte, triggering serum lysozyme activity, and resistance for coccidia infection. These functions increase immune responses during stress cycles and disease outbreaks in poultry and other animals. Additional research is recommended to elucidate the role of the host genetic makeup involvement in the relationship between methionine and other disease factors.

Keywords: Methionine; Immunopotentiation; Nutrition; Poultry

Abbreviations: Met- Methionine; AA- Amino Acid; Arg- Arginine; His- Histidine; Iso- Isoleucine; Leu- Leucine; Lys- Lysine; Phe- Phenylalanine; Thr- Threonine; Try- Tryptophan; Val- Valine; Pro- Proline; Cys- Cysteine; Gly- Glycine; EAA- Essential AA; NEAA- Nonessential AA; CEAA- Conditionally EAA; FAA- Functional AAs; SAA- Sulfur amino acids; TSAA- Total sulfur amino acids; CAS- Chemical Abstracts Service; RN- Registry Number; FDA- US Food and Drug Administration; UNII- Unlicensed National Information Infrastructure; SAMe- S-adenosylmethionine; MAT I- Methionine adenosyl transferase I; MAT II- Methionine adenosyl transferase II; SAH- S-adenosylhomocysteine; HCys- Homocysteine; SucCoA- Succinyl-CoA; CAC- Citric Acid Cycle; TCA-Tricarboxylic acid cycle; MS- Methionine synthase; BHMT, E.C.2.1.1.5-Betaine-homocysteine methyltransferase; DLM- D, L-methionine; MHA-FA- L-methionine hydroxy analog-free acid; MHAC-DL- methionine hydroxyl analog calcium; AAFCO- American Feed Control Officials; CFR-Code of Federal Regulations; FDA- Food and Drug Administration; BCAA- Branched-chain AA; GMOs- Genetically modified organisms; *E. coli- Escherichia coli*; *C. glutamicum- Corynebacterium glutamicum;* ME- Metabolic energy; CP- Crude protein; N- Nitrogen; NRC- National Research Council’s; ME:CP- Metabolic energy to crude protein ratio; AF- Abdominal fat; FC- Feed conversion; FI - Feed intake; BWG- Body weight gain; FE- Feed efficiency; EW- Egg weight; EP- Egg production; BW- Body weight; FCR- Feed conversion ratio; AFP- Abdominal fat pad; AME- Apparent metabolizable energy; DM- Dry matter; IP- Ideal protein; GI- Gastrointestinal tract; HI- Heat increment; ROS- Reactive oxygen species; AGP- α-1 acid glycoprotein; (IL)-l- Interleukin; LPS- Lipopolysaccharide; LMI- Leucocyte migration inhibition; PHA-P- Phaseolus vulgaris; IgG- Antibody immunoglobulin G; NDV-Newcastle disease virus; Ab- Antibody; SOD- Superoxide dismutase; GSH-Px- Glutathione peroxidase; MDA- Malondialdehyde; PHA- Phytohaemagglutinin; ConA- Concanamycin A; Th- T-lymphocyte helper cells; SRBC- Anti-sheep red blood cell antibody; CBH- Cutaneous basophilic hypersensitivity; CV- Coefficient of variation; HCT%- Hematocrit

1. Introduction

Amino acids (AA) are the building blocks of biological proteins. Over 500 AA exist in nature, though 20 of which can incorporate into proteins by arranging in myriad of ways (i.e metabolic proteins, structural proteins, enzymes, as well as precursors of multiple body constituents) serving variety of functions [1-3]. AA obtained from protein are utilized by avian species to fulfill a variety of function. Such that, proteins are underlying constituent of structural and protective tissues such as skin, feathers, ligaments, bone matrix, bone matrix, soft tissues, inclusive of organs as well as muscles [4].

The carbon skeleton of AA categorizes the dietary essentiality of AA [6]. According to the National

Research Council's (NRC), ten out of the twenty-two AA (arginine (Arg), histidine (His), isoleucine (Iso), leucine (Leu), lysine (Lys), Methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Try) and valine (Val) found in body proteins are classified as the essential AA (EAA) for the nutrient requirements of poultry [4]; that is, they are indispensable as their carbon skeleton cannot be synthesized de novo by the avian species. In contrast, AA that can be synthesized by the animal are known as nonessential AA (NEAA) [5-7]. It was assumed that animals do not require NEAA in their diets for maximal nutrition as they are capable of synthesizing adequate quantity of NEAA. Scientific research on animal as well as cell culture have nonetheless demonstrated evidence of NEAA carrying critical roles in multiple signaling pathways, while further demonstrating the importance of considering AA functions beyond their protein synthesis as young animals are unable to synthesize adequate amounts of NEAA particularly during early phase development to support their growth as the rates of utilization are relatively greater than rates of synthesis; to poultry this is true for Arg and proline (Pro). Such AAs are referred to as conditionally EAA (CEAA) [5, 8-10].

Functional AAs (FAA) is rather a notion developed to further define those AAs that regulate and engage in key metabolic pathways to improve health, survival, growth, development, and the reproduction of organisms. An observed deficiency in FAA whether it be EAA or NEAA hinders both the synthesis of protein as well as the species' body homeostasis [11].

Methionine is an EAA as it cannot be biologically synthesized by the bird and due to its exceptional emphasis on poultry growth and production [12]. Met is also classified as a FAA. Animals ought to obtain EAA from the diet, however many feed ingredients lack some of the EAA. In a typical corn-soybean meal, birds' requirement for fulfilling certain EAA may fail; this is the case for lysine (Lys), methionine (Met), threonine (Thr), and tryptophan (Trp). Met is considered the first limiting AA, with lysine and threonine being the second and third limiting AA respectively in a practical corn-soybean poultry fed diet [13, 14].

When formulating bird diet, sulfur amino acids (SAA) are crucial addition. Met and cysteine (Cys) are the two sulfur-containing AA (SAA); both are the principle providers of organic sulfur within the avian body and ought to be considered together as total sulfur amino acids (TSAA) nutrient requirements [15-19].

All AA must be present in cell into order for correct protein synthesis to take place. Therefore, it is crucial for protein and EAA to be supplied by the diet, where formulating dietary requirements for both ensures the appropriate approach for physiologically required AA to be implemented [7].

Met is ought to be available sufficiently in the diet to provide the building blocks of tissues, immune cells and to support the development of feathers [6, 20, 21]. Elevated levels of Met exist in eggs, sesame seeds, in addition to Brazilian nuts, fish meal, corn gluten meal, alfalfa meal, as well as sunflower seeds meal [20, 22-24]. Yet, Met is found to be limited in most natural sources of plant protein and it is therefore established as the first limiting AA for broilers and the second limiting AA for laying hens in a typical corn-soybean meal ration [24]. This article discusses the effect of immunopotentiation of methionine on broilers by going through methionine forms, metabolism, deficiency, and toxicity. It also shows the nutritional, fast-growing and slow-growing requirements in addition to the digestibility and the ideal protein ratio and choosing between cysteine and methionine as the Ideal sulfur amino acid in diets.

2. Methionine metabolism

Methionine possesses an empirical formula of C5H11NO2S, Chemical Abstracts Service (CAS) Registry Number (RN) 63-68-3; US Food and Drug Administration (FDA) Unlicensed National Information Infrastructure (UNII) with molecular structure demonstrated in Figure 1 [25, 26]. The sulfur-containing EAA has an asymmetric form, constructing both L- and D-isomers and the molecular structure of racemethionine is illustrated in Figure 2 below. The liver is the main site of Met metabolism where Met is the first limiting AA in commercial poultry [27]. The metabolism of Met is vital for numerous physiological processes (Figure 3). Expressly, Met is principally involved in the aid of methyl transfer reactions via its conversion to its active form as S-adenosylmethionine (SAMe) which is achieved by the catalyzation of methionine adenosyltransferase I (MAT I) and methionine adenosyltransferase II (MAT II) [10, 28]. The fact that vertebrates are unable to synthesize methyl groups which are essential constituents in their diets [29] proves Met methyl role a crucial one. Other than Met, potential dietary sources of methyl groups include choline, folic acid, and betaine [29]; however, they differentiate in their methylation availability and reactions. In the case of Met, it is necessary for protein synthesis. While as for instance with choline; it is primarily utilized in cell membranes as well as neurotransmitter [30, 31].

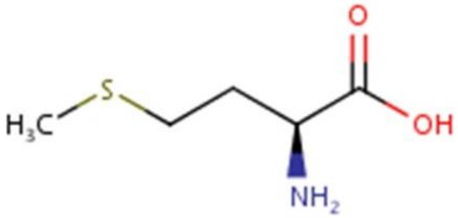

Figure. 1: Molecular structure of methionine, CAS RN: 63-68-3; UNII: AE28F7PNPL (ChemIDplus, 2017; FDA, 2017) [Diagram credit to ChemIDplus, 2017].

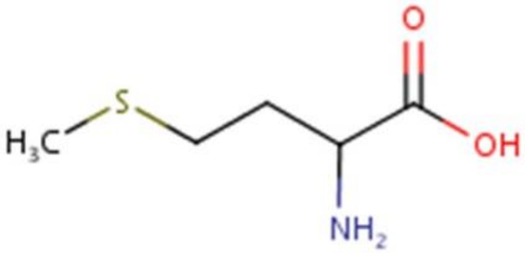

Figure. 2: Molecular structure of racemethionine, CAS RN: 59-51-8; UNII: 73JWT2K6T3 (ChemIDplus, 2017; FDA, 2017) [Diagram credit to ChemIDplus, 2017].

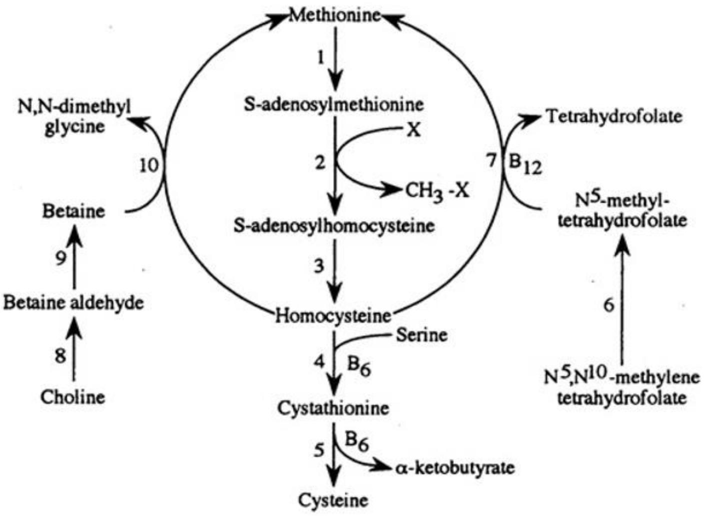

Figure. 3: Methionine metabolism. Enzymes depicted are as followed (1) methionine adenosyltrasferase, (2) various enzymes, (3) S-adenosylhomocysteine hydrolase (EC 2.1.1), (4) cystahionine β-synthase, (5) cystathionine γ-lyase, (6) α-ketoacid dehydrogenase, (7) N^5, N^{10}- methylenetetrahydrofolate reductase, (8) methionine synthase, (9) choline dehydrogenase, (10) betaine aldehyde dehydrogenase, and (11) betaine-homocysteine methyltransferase (EC 2.1.1.5) (Illustration adopted from Emmert *et al*., 1996).

Name	L-Methionine
IUPAC	L-2-amino-4-(methylthio)butyric acid, S-2-amino-4-(methylthio)butanoic acid, H-Met-OH
Formula	$C_5H_{11}NO_2S$
Molecular weight	149.208g/mol
Water solubility (20°C)	53g/L

Vapor Pressure	5.23E-07mm Hg (25°C)
Melting point	283 dec °C
***pKa*^{1} (-COOH)**	2.28 (25°C)
***pKa*^{2} (-NH3)**	9.21 (25°C)
Isolelectric point (*pI*)	5.74 (25°C)
Henry's Law constant	2.11E-11 atm-m3/mole (25°C)
pH (1% aqueous solution)	5.6-6.1

Table. 1: Chemical and Physical properties of Methionine[1]**.**

[1] Information and values credit to PubChem, 2017; ChemIDPlus, 2017.

Post its conversion in Met metabolism, same is next capable of behaving as a methyl-donor by releasing its terminal methyl group (and is the predominant methyl ($-CH_3$) donor in Met metabolism) of which can occur in an assortment of methyltransferase reactions (E.C. 2.1.1) [31]. Such reactions permit the synthesis of choline, creatine, epinephrine, glutathionine, lipoic acid, DNA as well as several other necessary compounds [32, 33]. Once SAMe has donated its methyl group, it is irreversibly converted to S-adenosylhomocysteine (SAH). Via the removal of an adenosine molecule, adenosylhomocysteinase converts SAH to homocysteine (HCys). Then there are two fates of HCys; it is either transsulfurated to Cys or remethylated to Met in Met metabolism [27].

Governed by the activity of Cystathionine β-Synthetase, HCys merges with serine constructing a molecule of cystathionine. This is broken down via cystathionine-γ-lyase into a molecule of Cys and a molecule of ∝-ketobutyrate [23, 31]. In both of these reactions, a co-factor of vitamin B6 is obligatory in the form of pyridoxal phosphate [34]. The ∝-ketobutyrate generated through this pathway is later converted to propionyl-CoA via multiple steps using ∝-ketoacid dehydrogenase. Succinyl-CoA (SucCoA), the end product metabolized from propionyl-CoA then enters the Citric Acid Cycle (CAC) (also known as tricarboxylic acid (TCA) cycle or the Krebs cycle) to generate energy [27, 31]. Thereby, two molecules of the regenerated cysteine will compose a single molecule of cysteine, that is the structural component of keratin; the leading protein present in skin, hair, nails, and feathers [23].

Alternatively using vitamin B12 as a cofactor; HCys can be remethylated to Met from N5- methyl-tetrahydrofolate by the route of methionine synthase (MS) [27, 34]. In addition, post its development by the oxidation of choline; homocysteine can also be remethylated to Met from betaine via betaine-homocysteine methyltransferase (BHMT, E.C.2.1.1.5) [27, 31, 35].

3. Forms of methionine

Mueller, in 1923 was the first to isolate Methionine from casein and since then much research has been towards the importance of Met in nutrition and animal feed [36]. AA, apart from glycine (Gly) [5, 21] are available in two forms which are referred to as D- or L enantiomers. In nature, Met is predominantly present in the L-form. While, the D-enantiomer is biologically inactive. Poultry are capable of utilizing both D- and L- forms of Met [37]. Both forms can be metabolized by a DL-racemase, which is needed for supplementing the chemically synthesized DL-methionine racemate as feed additive in livestock farming [38]. Met usually exists as a white crystalline powder. As a limiting AA, Met is supplemented in the form of dry D,L-methionine (DLM), racemic 2-amino-4-methylthiobutyric acid, with approximately 99% purity [38] , or liquid as either D,L-methionine hydroxy analog-free acid (MHA-FA) with equivalency of 88% racemic 2-amino-4-methylthiobutyric acid or DL- methionine hydroxyl analog calcium (MHAC) with 97% racemic 2-amino-4- methylthibutyric acid and calcium salt activity post-conversion of the analog into the active biological form [38] according to the Association of American Feed Control Officials (AAFCO) Code of Federal Regulations (CFR), Title 21CFR 582.5477 [39]. As α-keto acid analogs (the amine group replaced by a hydroxy group), MHA-FA and MHAC in avian species can be converted to the amino form via liver transamination applied by non-EAA (i.e. glutamic acid) [6]. DLM, MHA-FA, MHAC are all recognized as safe by the Food and Drug Administration (FDA) according to Title 21CFR 582.5475 and Title 21CFR 582.5477 [25, 39].

As mentioned earlier, it is not solely the level of methionine that is required but rather a balance of all EAA. Focusing only on methionine levels will generate AA imbalances and thus protein degeneration. Moreover, antagonistic relationships between certain AA have been present, an example of this exist between branched-chain AA (BCAA) leucine-isoleucine-valine, threonine and tryptophan and between arginine and lysine (37, 40].

Met is commercially synthesized via acrolein and methyl mercapton condensation [41, 42]. This compressed compound is then reacted with ammonia and hydrogen cyanide to generate a racemic mixture of the D and L isomers of Met which is effectively 100% pure [41]. The resulting compound is 1:1 ratio of D-Met and L- Met; where the racemic mixture DL-Met is a of 50% D-Met and 50% L-Met [38, 42].

Most of this manufactured Met is used for animal feed in livestock production amounting above 600,000 tons/year in 2013 according to the world market [38, 43]. This does not include organic farming due to the ban or high limitation on synthetic Met usage [20, 44]. Met has also been shown to be synthesized by bacterial fermentation [45-48] for up to 5 g/L with unemployment of genetically modified organisms (GMOs) [38]. The topmost concentration level of L-methionine via fermentation depicted at 35 g/L using a GMO of *Escherichia coli* (*E. coli*) [38]. Yet, to naturally synthesis Met, the process undergoes complex regulations including mutations, genetic modification, selection and optimization with only few strains of bacteria are capable of producing relevant amounts of Met, a major one being

Corynebacterium glutamicum (*C. glutamicum)* [38, 49, 50].

As to whether which of L-, D-, or D-L- Methionine is more efficiently absorbed, statistical methodology and research trials to evaluate the precise bioefficacy for D-, L-, DL-Met have estimated to be comparable [51-53].

4. Methionine requirement

4.1. Nutritional requirement

Birds eat to satisfy their metabolic energy (ME) requirement [7]. As with all animals, poultry require energy (carbohydrates, and fats), protein, vitamins, minerals, and water [54, 7]. Formulation of diet must meet the EAA and this ought to be relative to the level of ME. Several factors can affect dietary AA requirement and their utilization efficiency [55]. These include age gender, genetics, reproductive state, stage of production, ambient temperature, housing system, immunological stressors, production aim as well as metabolic energy, the level of proteins, and the availabilities of vitamins and minerals in the diet (4, 6, 7]. Poultry do not have specific requirements for crude protein (CP) per se; rather only for AA levels. Yet, CP should be present to meet the requirements of EAA, and enough nitrogen (N) to synthesize the non-EAA. Amino acid requirements are usually presented as percentages of the diet or may percentage of protein requirement. National Research Council's (NRC) *Nutrient Requirements of Poultry* is the fundamental reference for feed formulation. Dietary AA can be classified as both qualitative and quantitative [56]. Qualitative requirements rest under questions of "*what*" are the AA needed for maintenance, optimal performance, including growth, lactation and reproduction and optimal health including prevention of abnormalities, resistance, and the recovery to infectious diseases [5, 56]. Quantitative requirements follow questions of "*how much"* of an AA is needed for maintenance, optimal growth, and health [56]. The nutritional requirements of broilers are applied for starter, grower, and finisher phases as the requirements change with age; generally, with decrease in AA levels and an increase in ME. Overall CP contents of 23, 20, and 18% are applied for starter, grower, and finisher stages, respectively.

An unbalanced diet leads to poor poultry performance. In the case of deficient protein diet, i.e metabolic energy to crude protein (ME: CP) ratio; birds will overconsume energy to obtain sufficient protein [20]. Increasing the level of Met in broiler diets significantly reduces the abdominal fat (AF) content, this was reported by Summers and Leeson [57], whose work agreed with results by Mabray and Waldroup [58], demonstrating decreased abdominal fat (AF) weights as the levels of dietary Lys and Met increased. Deficient EAA diets can increase FI. According to Cherry & Siegel [59] pullets' diets equal in energy and contained 15% crude protein with only difference in levels of Met and SAA observed increase in FI to compensate for a marginal SAA deficiency, and that the SAA requirement for maximum feed conversion (FC) efficiency was higher than the requirement for egg production (EP).

Including ME and AA content in feed, feed intake (FI) could be influenced by nutritional value and toxicity of the feed, palatability, particle size and environmental temperature [7, 60]. With high metabolic energy diets, poultry FI decreases, and AA intakes are thus restricted [6, 61].

During heat stress (high ambient temperature and humidity), FI decreases, evoking reduced growth and egg production, poor performance, leading to physiological and immunological stress, susceptibility to disease, reduced welfare status and high mortality rates. This results in detrimental effects of reduced welfare status of birds and economic losses to poultry production [62].

In period of heat waves, it is important to implement a combination of methods to aid in alleviating heat stress, ranging from housing, management, and feeding practices [62]. An additional method is nutritional manipulation. Administering high AA and high protein diets to birds during high ambient temperature demonstrated negative impact body weight gain (BWG), feed efficiency (FE), and carcass composition and yield [62, 63]. AA supplementation may partially prevent growth depression of heat stressed flocks, yet it is supplementation of low-apparent metabolizable energy (AME) and high AA diets at high temperature that significantly decreases the bird’s FI, BW, absolute carcass, breast, wing, front half, including back half weights [7, 55, 60].

Universally recognized as the first limiting AA in broilers, methionine is also the second limiting AA for laying hens fed on practical corn-soybean meal diets [7, 64]. NRC (1994) requirement for methionine are 0.50, 0.38 and 0.32% for starter (0-3 weeks of age), grower (3-6 weeks of age) and finisher (6-8 weeks of age) broiler phases respectively; with 90% dry matter (DM) basis of 0.90, 0.72, and 0.60% total TSAA requirement, respectively.

If the NRC requirement is not met for any EAA, the efficiency of poultry production is reduced immensely with great losses in broiler growth and egg size in laying hens [20, 64, 65]. Therefore, synthetic Met in form DLM, MHA-FA or MHAC are generally supplemented in poultry feed to meet their dietary requirements. It is crucial to adjust the concentration of all nutrients in diet in relation to the level of metabolic energy to provide a nutrient balanced diet. Thus, in the case of increased ME, Met requirement increases [66], and it is recommended to prepare poultry feed with AA needs calculated as percentage of ME. [67] observed that as wider the ratio of ME to protein tended to be, broiler consumed greater energy and deposited greater fat and less water in their carcasses. The present-day commercial bird is very distinct from commercial birds prior to 1991 [68, 69]. Some research suggests that AA requirement today differ greatly from those highlighted by the NRC (1994) as to reasons of genetic selection, management practice and feed-related alterations [68]. Other studies state that increasing levels of Met ought to be above the NRC (1994) recommendations [70, 71]. Further, studies by [72-74], report that methionine requirements for optimal immunity are higher than for optimal growth.

4.2. Fast-growing versus slow-growing broilers requirements

Dietary AA concentrations ought to match the needs for both maintenance and skeletal muscles [75]. The requirement of AA of fast-growing broiler breeds may be greater than slow-growing breeds. This is coherent with higher protein to fat ratio of fast-growing genotypes than slow-growing genotypes [20, 76]. Thus, higher AA to ME ratio is required for faster growing breeds [77]. As expected, the rapid growth rate of broiler today requires increased contents of nutrients and ME daily, however these demands for different nutrients are not in the same proportions as previously stated [77, 78].

Met requirement of fast-feathering versus slow-feathering genotypes has been indicated to be the same of Met level of 0.46% and 0.46% respectively for optimized nitrogen retention and 0.50% and 0.50% respectively for optimized version (FC) during the grower phase [79, 80]. As a sulfur AA and compared to Met, Cys requirement for fast and slow growing strain illustrated to be lower, with 0.44% and 0.39% respectively [79].

Methionine and total sulfur amino acid requirements for broilers with fast, medium, and slow growing genotypes have demonstrated to be analogous in the starter and grower phases, [7, 81, 82]. Increasing graded level of Met in basal diets has significantly increase BWG, but no interaction has been illustrated between the Met content and broiler genotype [20]. Yet an interaction was evident taking into consideration the measurement of breast yield. Breast yield of fast-growing broilers responded to increasing dietary content of Met, breast yield of medium-growing breeds responded solely to the intermediate content of Met, and breast yield of the slow-growing breeds responded solely to the diets with higher content of Met [20, 83].

5. Digestibility and ideal protein ratio

An ideal protein (IP) is one that consists of the explicit amounts of AA necessary for the animal without deficiencies or excesses [84]. No particular assortment of AA requirements is assigned to any animal following conditions of age, gender, body composition and nutrition combined [56]. The concept of IP holds a mixture of EAA that explicitly match protein accretion and maintenance demand i.e meeting the animal’s need for its specific growth stage or level of production, without under- or over-feeding of AA [22]. In order to determine the ideal amino acid ratio, it is mandatory to know the digestible amino acid requirement of each EAA for the chicken and its relationship to lysine [85]. IP ratios (AA-to-Lys ratios) are expressed as percentages of digestible Lys established on digestible AA requirements rather than TAA requirements [86]. This means that to consider Met requirement, it is crucial to focus on Met as well as a balanced profile of all the EAA [2, 86]. For this reason, the concept of IP is employed in diet formulation to aid in balancing and supplying with greater precision all the EAA in addition to the NEAA (such as glutamine, glutamate, proline, glycine, and arginine which are influential in regulating gene expression, cell signaling, anti-oxidative responses, fertility, neurotransmission, and immunity) for ideal performance and increased profitability [22, 56, 62].

Poultry possess very short digestive tract of which is particularly sensitive to pH alterations. Few natural crude proteins are gradually digested, thus the available AA that can be absorbed and deaminized prior to those that are gradually released are available for absorption. The liver however is not capable of storing AA, this means that if the AA are not absorbed, when necessary, they cannot be utilized for protein synthesis [87]. Several factors may influence protein digestibility. Yet AA content and chemical analysis may demonstrate a complete essential AA profile; factors as solubility, structure and type of proteins can affect digestibility [87].

Regular assays for requirements are not satisfactory. This is due to the factors affecting AA requirements: dietary ME or CP levels, age of birds, genetics, and gender. Thus, it is impossible to address all these factors in one trial, taking into consideration individual AA. This is the reason for a need of ideal ratios with Lys used as reference AA. The justifications for using Lys as a reference for ideal protein is 1) Lys is the second limiting AA in poultry diet, and in fact supplementing a limiting AA (i.e. methionine and lysine) to poultry diets increases the efficiency of protein utilization, and in turn N excretion will be reduced; 2) Lys is easier to analyze than Met or Cys, 3) Lys is almost exclusively used for body protein and thus not complicated by pathways related to maintenance and feathering 4) Ample of data for the digestible Lys requirement of poultry are available, and 5) Lys requirement for several dietary, environmental, and body compositional circumstances are readily available [7, 61, 88-90].

Yet, the NRC (1994) lists the TAA requirement as opposed to levels of digestible AA; and in ingredients of practical plant based diet, the content of AA is not equal to the available AA content for the presence of anti-nutritional factors [89, 91]. To determine the digestibility of feed ingredients, the below equation is used. This classical method for evaluating feed ingredients where measurements of digestibility allow for determining the amount of a certain nutrient absorbed in gastrointestinal tract (GI) from a given quantity of food. For instance, the % digestibility of protein is calculated as:

(DW diet eaten × %Pro diet – DW feces voided × %Pro feces) / DW diet eaten × %Pro diet
Whereby: DW goes for “Dry weight of”, Pro goes for “Protein in”.

The similar equation may be utilized to determine the percentage of digestibility of CP, fat, dry matter, energy, or any other nutrient [7].

The use of an ideal AA ratio may aid in decreasing feed costs. Diets formulated on a digestible basis have illustrated to provide augmented performance when compared to diets formulated on a total amino acid basis [85, 92].

Note that proteins of those which supply solely the desired level of essential AA are preferred to those which provide a high excess of some essential with minimum level of another essential. This is due to the fact that large surplus of one type of AA can be antagonistic to another [87]. This antagonism relationship among AA particularly occurs between or among AAs belonging to the same group, such as Lys and Arg, or branched chain AA (Leu, Ile and Val) [40]. In such case increasing the level of one AA above its requirement necessitates increasing the level of the other AA [40]. It is important to highlight that this experimental thesis is aware and considers the antagonist relationship between AA as well as the previous research done on high excess of methionine demonstrating its negative turn out [65, 93-95]. This is why within this thesis experiment methionine is not supplemented in high excess but rather slightly above nutritional requirements for poultry.

It is also important to note that the utilization efficiencies of individual EAA are different [21, 96].

6. Choosing between cysteine and methionine as the ideal sulfur amino acid in diets

The reason for Met to be perceived as an ideal SAA than Cys is due mainly to the fact that Met is an EAA and Cys is not. In addition, TSAA requirement can be provided solely by the metabolism of Met. This is achieved via Met transsulfuration pathway where Met serves as a precursor of Cys. In contrast, Cys does not serve as the precursor of Met due to the irreversibility of Met transsulfuration pathway [27, 97-99]. Yet, studies on Met-sparing effect of Cys [97-99] where Met utilized in transsulfuration pathway was replaced with Cys demonstrated to be inadequate. This is because a raise dietary Cys and a reduction in dietary Met consequently raise dietary organic sulfur at the same concentration of TSAA, which means Met-sparing experiments may alter the quantity of organic sulfur in TSAA [100, 101].

7. Methionine deficiency

Malnutrition and infections are major obstacles to survival, growth, reproduction, and health [102, 104]. Dietary AA deficiency hinder concentrations of majority of AA found in plasma while damaging the lymphatic system [104, 105].

According to Elwinger and Tausen [106] reduced MET levels decreased feather cover and egg weight (EW), though egg production (EP) was not affected. Futher, they observed that FI increased as feather cover deteriorated, hence a reduction in feed efficient (FE) was apparent. Met deficiency in poultry is presented with reduced growth, performance, FI, BWG, FCR, breast meat yield, and increased abdominal fat pad (AFP) deposition [107, 108]. Met-deficient diet induces reduced growth and performance as feed lacking adequate level of Met to accommodate for maintenance, growth and production of poultry prompts for poor growth rate, FCR, BW, FCR, egg size and production for layers and breeders [20, 109]. As Met is a SAA and sulfur is a major constituent of feathers, Met-deficient diet is associated with poor feather development. Bird with met deficiency is likely to feed on feathers in an effort to satisfy a craving for Met and as such feather picking can lead to cannibalism circumstances in a flock or lead the bird to pick on its own feathers resulting in higher incidence and/or severity of bacterial infections [110, 111]. Further SAA execute antioxidant functions in the avian body preventing destruction of cells. Rubin et al [112] have reported that higher levels SAA may be beneficial to resilience to diseases. Swain and Johri, (2000) demonstrated Met incorporation of (0, 1.5, 2.0, and 4.5 g/kg diet) significantly augmented ($P < 0.05$) the cellular immune response. Avian met deficiency can well lead to a flawed lymphatic system, high morbidity, and mortality due to debilitated mechanisms of T and B lymphocytes [72].

Not incorporating Met to diet formulation leads to elevated levels of CP and unbalanced IP ratios, leaving excesses of AA behind unemployed for growth, production, etc. that ought to be metabolized and excreted. This therefore propagates incidence of kidney disorders and elevated heat increment (HI). Unable to cope with high HI, birds undergo HS particularly in warm regions establishing high mortality rates [62, 113]. On the other hand, the supplementation of Met aids in lowering the pH level of urine via excretion of sulfate anion and thus impeding the development of kidney stones, uroliths, or urologic syndromes [114].

8. Methionine toxicity

High levels of Met have been indicated to be toxic for avian species, this is in accordance with [93, 115-117] stated that the high excess of DLM or DL-HMB-FA Met supplementation reduced WG and FI of ducks significantly. Particularly growth depression was observed in broilers as well as turkeys when Met content in diet was supplemented above 1% [116-119]. It is worth noting that high levels of plasma homocysteine (HCys) are suggested to be an index of overage dietary Met considering HCys is a precursor for Met synthesis and a metabolite of Met degradation [27, 120].

9. Immunopotentiating function of methionine

As an essential and first-limiting amino acid, Methionine is suggested to play an influential role in both humoral and cell-mediated immune responses. Amino acids are required for clonal proliferation of lymphocytes; the delivering of new bone marrow monocytes and heterocytes and synthesis of effector molecules (immunoglobulins, lysozyme, nitric oxide, complement); formation of bursa of Fabricius' germinative centers to perfect immunoglobulin affinity; and the development of communication molecules (such as cytokines and eicosanoids) [65, 112]. Multiple functions are dependent upon Methionine, however its notable roles include 1) protein synthesis; 2) precursor for glutathione; a tripeptide that curtails reactive oxygen species (ROS) safeguarding cells from oxidative stress; 3) methionine is required for the synthesis of spermine and spermidine which are polyamines that engage in nucleus and cell division; and 4) methionine is a vital methyl donor, methylating the reaction of DNA as well as several distinct molecules [5, 75, 112, 121].

Considerable scientific research studies have demonstrated methionine protagonistic interference in the immune system of poultry, leading to an enhancement in both humoral and cellular responses. One reason for such effect is employed by the intracellular glutathione and cysteine levels [112]. Immune cells proliferation is sensitive to intracellular disparities in glutathione and cysteine levels which are also involved in the metabolism of methionine [73]. Glutathione, carrying multiple important activities is known as the most abundant intracellular antioxidant compound and is crucial for the protection against the emergence of oxidative stress occurring posts inflammatory processes [112, 122]. For protein synthesis to occur in immune cells, sufficient dietary intake of both methionine and cysteine [sulfur-containing amino acids (SAA)] is important [123].

A study by Takahashi et al. [124] illustrated both Methionine and Cysteine (SAA) exerting beneficial aspect on immune and inflammatory responses to stress induced Escherichia *coli* lipopolysaccharide injection, and concanavalin A in male broiler chickens. Results indicated plasma α-1 acid glycoprotein (AGP) concentration and interleukin (IL)-l-like activity in chicks fed on the SAA- sufficient diet were higher following a single e injection of lipopolysaccharide (LPS) than those in chicks fed on the SAA- deficient diet [124].

Swain and Johri (2000) indicated that cellular immune response measured as leucocyte migration inhibition (LMI) increased significantly ($P< 0{\cdot}05$) at supplemented concentrations of methionine in broiler diets at 21 d of age demonstrating enhanced immunity [74]. Such observation was in conformity with study by Tsiagbe et al. [72], that revealed enhanced mitogen stimulation by Phaseolus vulgaris (PHA-P) as responses to phytohaemagglutinin and significant increase in total antibody immunoglobulin G (IgG) in chicks fed on corn-soybean diets supplemented with methionine [72]. Increased methionine is reported to be critical for the synthesis of the IgG necessary for Th cells function [72].

Experiment carried out to study Methionine deficient diet in challenged chicks with infectious bursal disease demonstrated significant decrease ($P<0.05$) in monocyte ratio and blood triglyceride; in addition to protein efficiency ratio, body weight gain, and feed conversion ratio [125].

Moreover, dietary supplementation with Methionine or Cysteine was beneficial for the lymphatic system under various catabolic conditions. In chickens challenged Newcastle disease virus (NDV) increasing dietary levels of Methionine (from 0.4 to 0.6, 1.2 and 1.8% respectively) in diets noticeably augmented T-lymphocyte proliferation in response to mitogen stimulation as well as IgG plasma levels [126]. While, an increased level of dietary Cysteine (from 0.185 to 0.37%) has shown similar effects as Methionine. However, high supplemental levels of Methionine and Cysteine (1.8 and 0.37%, respectively) were inimical to the chickens' performance and immune responses. This can be explained by the excess production of highly toxic elements such as homocysteine and sulfuric acid [65, 95] and thus a higher Cysteine content supplemented in poultry diet is considered to be toxic [65, 127]. While, lower sulfur-containing amino acid levels have resulted in a severe lymphocyte depletion in the Peyer's patches and in the lamina propria [74, 112].

Hence, it is crucial to remark that neither the excess nor the deficiency of methionine in diets influence the generation of primary antibodies in chickens [65, 74, 128, 129]. Moreover, according to Rubin *et al*., (2007) vaccines administered on 1-day of age can impair the bird's performance up to 21 days of age. Thus, it is recommended to carefully administer vaccines, considering the risk of mortality caused by disease as compared to mortality caused by vaccines [130].

Majority of experimental studies conducted have focused on the effect of methionine deficiency on selected immune mechanisms in chickens [74, 112, 125, 131], and only a few researches investigated the influence of methionine on lymphocytes in peripheral blood and lymphoid organs in broilers [93, 115, 132].

According to [93] methionine deficient diet in broilers caused ultrastructural pathological changes in the thymus, decreased T-lymphocyte populations, reduced serum concentrations of interleukine-2 and T-lymphocyte proliferation via increase in the percentage of apoptotic cells. In another study, Wu *et al*., 2013 cited relative decrease in weights of the thymus and bursa of Fabricius as well as decrease in proliferative of thymocytes and bursal cells with lower levels methionine in diet than recommended by NRC (1994).

Thus, methionine deficiency can lead to lymphoid organs dysplasia [115, 133, 134], and decrease the relative weight of thymus, spleen and bursa of Fabricius [132, 135].

With regards to immune cell response, low level of methionine cause receding tubercularization reaction which proposes decline in Th1 lymphocytes proliferation within inflammation sites [130]. Such results were compatible with [74, 136] displaying levels of methionine below 0.50% spawn feeble immune response as distinguished to higher concentrations [74, 130, 136].

Methionine deficiency can significantly inhibit the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and the prevention of hydroxyl radicals while augmenting malondialdehyde (MDA) levels [137]. Further, methionine deficiency causes oxidative stress and lipid peroxidation, forcing the aggregation of free radicals, and hence destroying biofilm structure of lymphocytes. It also greatly hinders the reactivity of phytohaemagglutinin (PHA), the stimulus reaction of spleen lymphocytes to concanamycin A (ConA) as well as the mitogenesis of thymic cells [137, 138].

Histological studies acknowledge congestion in cortex and medulla of thymic lobule with loosely arranged and significantly reduced quantity of lymphocytes in the medulla in methionine deficient formulated diet [93]. Decline in lymphocytes were also observed in lymphoid follicles with thinner cortices and wider medullae in the bursa of Fabricius [135]. The histological structure of spleen was disarranged, and its lymphocytes were significantly decreased in the white and red pulp [135]. Vacuolated mitochondria of lymphocytes and greater apoptotic lymphocytes were detected in the spleens of broiler on methionine deficient ration [93, 132, 137, 138].

Commercial broiler chickens do not require above 0.50% and 0.38% Methionine in starter and grower diets, respectively for optimum growth and feed efficiency, while it has been observed that higher incorporation rates of Methionine are necessary to prompt immune responses [65, 73, 74, 139].

Elevated Methionine content, above the required dose for optimal growth, augments the immune response through both direct effects (protein synthesis and breakdown) and indirect effects involving Methionine derivatives. Few studies show the effects of methionine on nonspecific immune function as stimulating phagocytic activity of the leukocyte [140, 141], peripheral blood lymphocyte activity, as well as serum lysozyme activity [98, 142]. As with methionine derivatives, methionine is a substrate for the synthesis of choline and therefore resulted compounds phosphatidylcholine and acetylcholine play essential role in leucocyte metabolism and nerve function [127, 143]. Furthermore, Methionine has demonstrated important physiological function in regard to detoxification [143] and resistance for coccidium infection [136].

Antibody titers (IgG) in broilers supplemented with 1.2 and 0.9% methionine diet were significantly higher than those of low levels of methionine [144]. Such experiment illustrated significant increase of total leukocyte, percentage of lymphocytes and heterophils as well as significant weight changes of bursa and spleen. Further, increase body weights and higher feed intake were obtained at high methionine and low energy diet [144].

Turkeys that received methionine supplemented diet with at 5.98 g/kg; only slightly (by 8.7%) above the NCR (1994) recommendations have shown increase percentage in IgM+ B-cell subpopulation in the spleen [145].

Also, optimizing leukocyte migration inhibition assay required greater content of methionine than the growth promoting level in broiler chicks [146]. Similarly, higher methionine was required for antibody response and thymus derived T-lymphocyte helper cells (Th) function in full-feathered broiler, in addition to a higher leucocyte migration inhibition value and enhanced antibody titer of New Castle Disease virus in full-feathered broilers [147].

The results of recent experiments on poultry are insufficient to define the optimal dietary levels of Methionine for sparking immunoexertory activity. However, a study by Rama Rao *et al.* (2003) have illustrate an elevated anti-sheep red blood cell (SRBC) antibody titers and greater cutaneous basophilic hypersensitivity (CBH) response with higher supplemented methionine diet and that the optimal methionine concentration for antibody production was the highest at 0.55% [136]. The SRBC administration pathway, genetic traits and gender may have influenced antibody production. However, in this study, the female birds of four different genetic strains were used, and SRBC was intravenously injected [136]. Similar results were observed in different study where male Ross broilers were used and SRBC was injected in the muscle [65, 112].

Findings illustrated that methionine can increase the relative weight of bursa of Fabricius and spleen in chicken [138, 144, 148] and while it can also increase the weight of thymus and bursa of Fabricius of layers in the brood rearing stage, it shows no significant effect on the spleen during this stage [131]. So, it is concluded that the development of the primary lymphoid organs: thymus and bursa of Fabricius possibly be easily influenced than the secondary lymphoid organs such as the spleen by methionine [134, 138, 149, 150].

Appropriate methionine concentration can significantly increase the degree of antibody and the sheep red blood cells antibody titer as previously explained and cited by the following authors [128, 129, 144, 147]. Methionine is capable of encouraging T-lymphocyte proliferation of the peripheral blood, thymus, and spleen, while methionine deficiency diminishes the transformation of T-lymphocyte proliferation [151]. It has also been evident that with higher levels of methionine in ration, serum antibody rises in the broilers with coccidium infection [138, 151], and as several research have pointed out the leukocyte migration and antibody titer also have increased in chickens infected by Newcastle disease virus (NDV) [74, 152].

On the other hand, Wu *et al.* (2012) reported that methionine deficiency can significantly decrease serum IgG, IgA and IgM content which in turn suggests that the humoral immunity is jeopardized [93]. [153] illustrated methionine's ability to significantly affect the content of serum IgM and IgA in a meat rabbit.

Deficiency in methionine influences the relative percentage of T-lymphocyte subsets which includes $CD3^+$, $CD3^+$ $CD8^+$ and $CD3^+$ $CD4^+$ of broilers [93].

Recent study by Ramadan et al [154] demonstrated that 20% excess methionine above modern broiler requirement significantly increased average body weight (BW) at 10 days of age; with no significant differences in BW and feed conversion ratio (FCR) at 17 and 35 days of age [154]. In *Mycoplasma gallisepticum*-challenged birds, the 20% excess methionine treatment significantly increased IgG titers (3170) in comparison to adequate methionine level (1843) along with coefficient of variation (CV) of 14.84 and 66.38%, respectively [154]. Results of Ramadan et al. (2018) also illustrated excess methionine had significantly increased bursal indices in *Mycoplasma gallisepticum* -challenged broilers at 35 days of age and augmented hematological parameters with a significant increase of hematocrit (HCT%) compared to tilmicosin-based antimicrobial that significantly decreased HCT%. Further, excess methionine significantly decreased the percentage of birds with severe tracheatis caused by *Mycoplasma gallisepticum*-infection from 40 to 10% at 17 to 35 days of age [154].

In addition, the level of the methionine is found to be higher in the immune response than in normal circumstance [130, 138] deducing that there exists a close relationship between the methionine and immune response or disease resistance [112, 130, 138].

10. Conclusion

1. Such substantiated extensive research review on methionine proves its multiple roles in poultry physiology and vitally highlights its immunopotentiating function.
2. Understanding the underlying mechanisms and metabolism of methionine and the process by which it alleges an impact on the lymphatic system is essential in decoding complex interactions between nutrition and disease.
3. The treatment or resistance against an infection necessitates an augmented response congregated by the immune system. From nutritional point of view, amino acids are required to stimulate an immune response including, but not limited to, lymphocytes proliferation, germinal centers enactment in the bursa of Fabricius, immunoglobulins affinity, synthesis of effector B-lymphocytes, nitric oxide, lysozyme, complement in addition to cytokines and eicosanoids. Several points were emphasized on methionine's role as an immunopotentiator; however, additional

research is also recommended to elucidate the precise genetic makeup involvement in the relationship between methionine and other disease factors.

4. It is important to note that albeit the indispensable function of cell-mediated immunity, the intervention of methionine, as a sulfur donor, on diseases may heavily rely on humoral immunity due to its role in the disulfide-bridge linkage formation in immunoglobulins.

Conflicts of Interest

The authors declare that they have no competing interests or personal relationships that could have influenced the work reported in this paper.

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