AMINO ACIDS

L-methionine, the best source of methionine for poultry and swine

Ramalho Rodrigueiro

Feed Brazil

Abstract

Methionine source for the best nutrition for poultry and swine requires not only understanding the concept of bioefficacy but also understanding how methionine sources can meet the physiological aspects of young and adult animals. This article aims to clarify why L-Methionine is the preferred source of methionine for animal nutrition.

Introduction

Protein is a structural nutrient made up of amino acids. It is known that there are several amino acids that have been identified, however, only 20 are used for protein synthesis. Clearly, 10 of these 20 amino acids can be synthesized endogenously on amounts needed to fulfill the animals' requirement for optimum performance (non-essential amino acids), whereas the other 10 amino acids must be consumed by the diet, hence considered as essential.

Methionine is a sulfur amino acid, as it contains sulfur in its chemical structure, which none of the other 10 essential amino acids involved in the formation of animal protein have (Oliveira 2014). It is an essential amino acid, being, respectively, the first and second limiting for poultry and swine when fed diets based on corn and soybean meal. In addition to protein synthesis, methionine plays an important role as methyl group donor for the synthesis of numerous components, allowing the formation of choline, creatine, epinephrine, glutathione (GSH), taurine, cysteine and polyamines. The functional effect of methionine is important for animal development and health (Burley et al., 2016; Shen et al., 2015). Methionine is also known to improve lipid metabolism and its positive impact on the immune system (Figure 1).

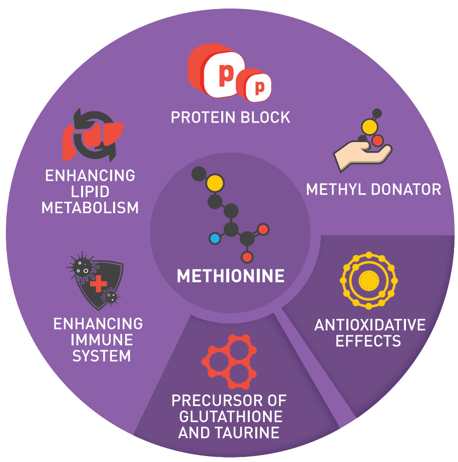

Figure 1. Physiological effect of methionine in the animal body.

The common sources of methionine in feed application, other than from intact feedstuffs, are DL-methionine (crystalline form and 99% purity) and DL-methionine hydroxy analogous (MHA), also known as 2-hydroxy-4-(methylthio) butanoic acid (HMTBA 88%), a low pH aqueous solution. Both products come from chemical syntheses and are dependent on petroleum derivatives.
DL-methionine was initially developed in 1936 but was only commercialized in 1945. Then in 1979, liquid DL-MHA was launched. They were recognized to be unique sources of methionine until 2015 when, another source of methionine was introduced in the global market as the first methionine commercially synthesized from fermentation process and acknowledged as L-methionine. Unlike other sources of methionine, L-methionine is considered a biologically functional (bioactive) source of methionine, as it is an amino acid that has 100% of its isomeric configuration in the levorotatory (L) form. Its fermentation process fits into the Eco-Friendly system with base for sustainability (Figure 2).

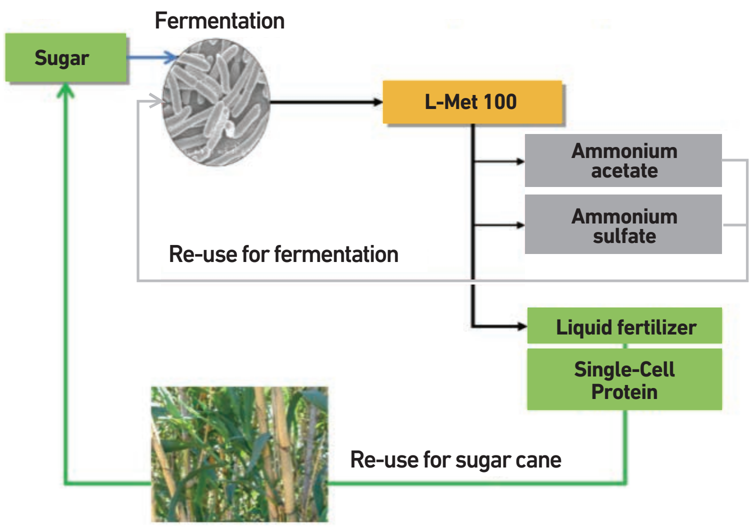

Figure 2. Eco-Friendly sustainability-oriented system for L-methionine

The synthesis of L-Methionine based on "zero level" aggression to the environment is due to the fact that sugar cane and corn are the main raw materials used in the industrial process. The residues from L-methionine fermentation become reusable in sugar cane and corn crops as shown in Figure 2.

As the L-methionine is in bioactive form, brings evidence that this amino acid has a peculiar characteristic in relation to other sources of methionine. The purpose of this article is to demonstrate the reasons that lead L-Methionine to be considered the best source of methionine for animal nutrition.

Importance of enantiomers in choosing the source of methionine

The nature of a chemical component can change depending on how the functional group is attached to the same molecule. Enantiomers, for example, are optically active and asymmetric space isomers. They are mirror images of each other and cannot be superimposed either by rotation or translation, so, if we place one molecule on top of another, they will not be the same, the arrangement of their binding atoms will be different. These enantiomers are called chiral molecules, (meaning "hand", as hands are asymmetrical and non-overlapping). The activity that each enantiomer exerts in the organism is distinct from one another. Right-handed (D) is often less active than left-handed (L).

The chemical processes employed on the production of traditional methionine sources produce two types of isomeric configurations representing the right-hand (D) and the left-hand (L). In the chemical process it is expected to obtain 50% D-methionine and 50% L-methionine (Figure 3) on crystalline product (DL-methionine) or 50% D-MHA and 50% L-MHA for the liquid product (DL-MHA) thus denominating mixture of enantiomers or racemic mixture.

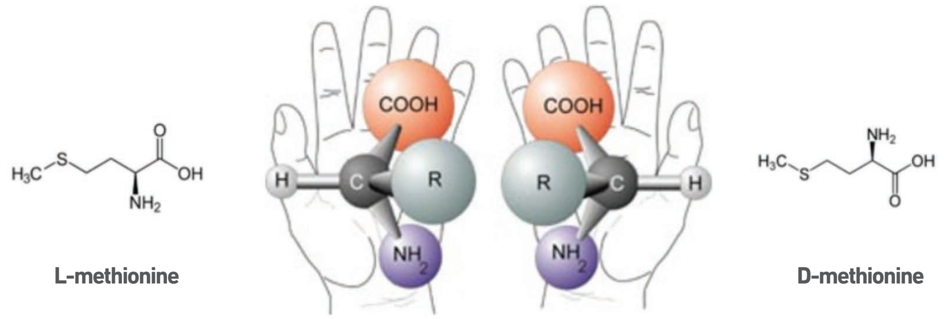

Figure 3. **Exemple of enantiomers**

The discussion about the bioefficacy values between DL-methionine (DLM) and DL-MHA is wide in the literatures. Regardless of the physiological mechanisms or statistical tools used to establish a bioefficacy value, it must be remembered that both sources of methionine (DLM and DL-MHA) are in the forms of D and L-isomers. For Leeson and Summers (2001), for many amino acids, the absorption occurs through an active process and against a concentration gradient. This transport mechanism requires energy expenditure and demonstrates specificity for the L form of amino acid. The D form is generally absorbed more slowly than the corresponding L form. The D-isomer form needs to be converted in the liver, kidneys and duodenum into its respective L-isomer via D-aminoacid oxidase and transaminase (Zhang et al., 2018). Therefore, D-Met is not directly used by cells, but needs to be converted to the L form (Shen et al., 2014). The steps to understand the isomeric effectiveness of methionine sources are shown in Figure 4.

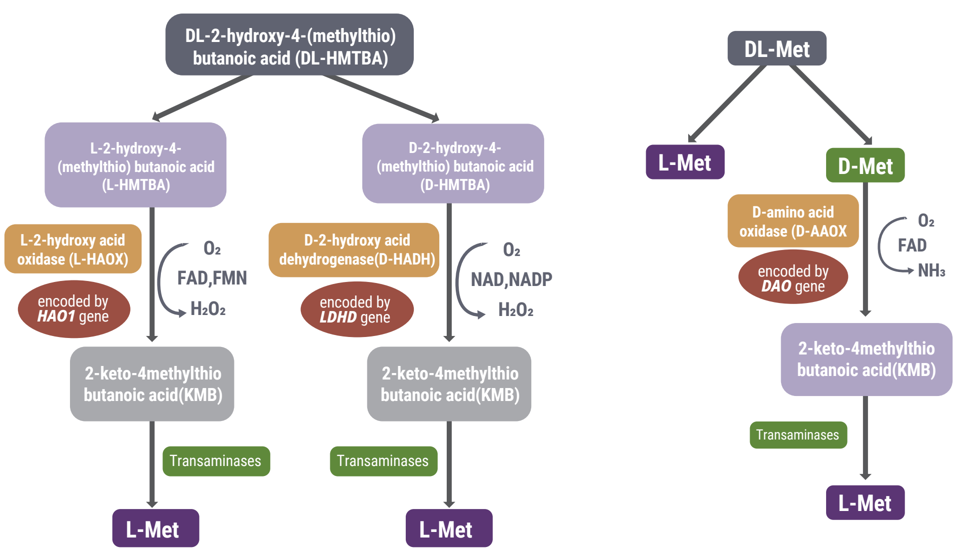

Figure 4. **Metabolic step in the isomeric effectiveness of methionine sources (Zhang et al., 2018).**

Importance of L-methionine in young animal nutrition

Understanding the importance of the development and physiological maturity of the gastrointestinal tract as well as the immune system of young animals is a crucial point in designing a diet. Therefore, this diet should stimulate the development of the digestive, enzyme and immune systems of neonates. According to Noy and Sklan (1998) and Stringhini et al. (2016), in poultries, for example, it is known that in the period close to and after hatch dramatic changes occur in intestine size and its morphology.

In the immediately posthatch period the intestine increases in weight more rapidly than body mass as a whole. This relative growth reaches a maximum of 4 to 8 days in chicks. Microscopic examination of the mucosa indicates that villus height and its area increase rapidly at different rates in various chick intestinal segments, reaching at 6 to 8 days in the duodenum and after 10 days in the jejunum and ileum.

The facts show that, in the nutrition of young animals, a large part of the nutrients are destined for the development of the intestine and other internal organs. For Chen et al. (2014), not unlike other amino acids, methionine affects intestinal protein metabolism. The gastrointestinal tract is a significant site of body metabolism, reaching 20% of dietary methionine consumed.

The splenic metabolism of methionine indicates that the gastrointestinal tract has a high requirement for methionine. D-Methionine is not used directly by intestinal cells until its convertion to L-methionine in the liver and kidneys. On the other hand, L-methionine, when administered via animal feed, will directly benefit the gastrointestinal tract and in the synthesis of glutathione (GSH), the main cellular antioxidant in the intestine of piglets and chicks (Figure 5, 6 and 7).

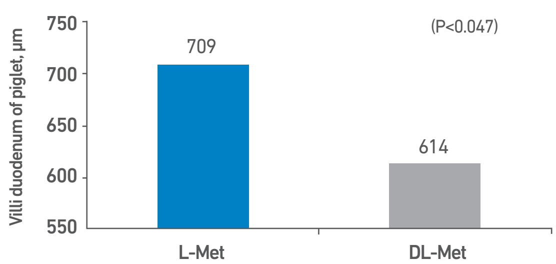

Figure 5. **Villi size in the duodenum of piglets fed a diet with L-methionine and DL-methionine (adapted from Shen et al., 2014).**

Information from Shen et al. (2014)
. L-met: 0.16% of met from basal diet + 0.145% L-met
. DL-met: 0.16% of met from basal diet + 0.145% DL-met
. Requirement 95% of NRC (1998).
. n = 10 per treatment.
. Weaning = 21 days and initial weight = 8.40±0.25 kg.
. Experimental period = 20 days after weaning.

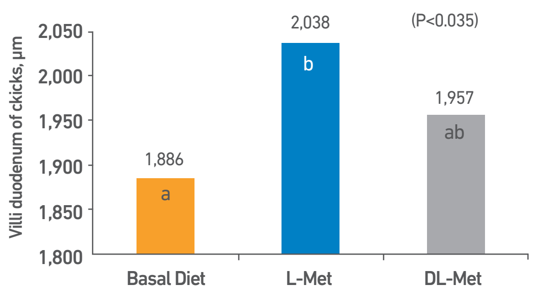

Figure 6. **Villi size in the duodenum of broiler chickens at 21 days of age when fed a diet with L-methionine and DL-methionine (adapted from Shen et al., 2015).**

Information from Shen et al. (2015):
. L-met: 0.56% of met from basal diet + 0.285% L-met
. DL-met: 0.56% of met from basal diet + 0.285% DL-met
. n = 11 per treatment.
. Analysis 7; 14 and 21 days of age
. Requirement 90% of Aviagen (2007).

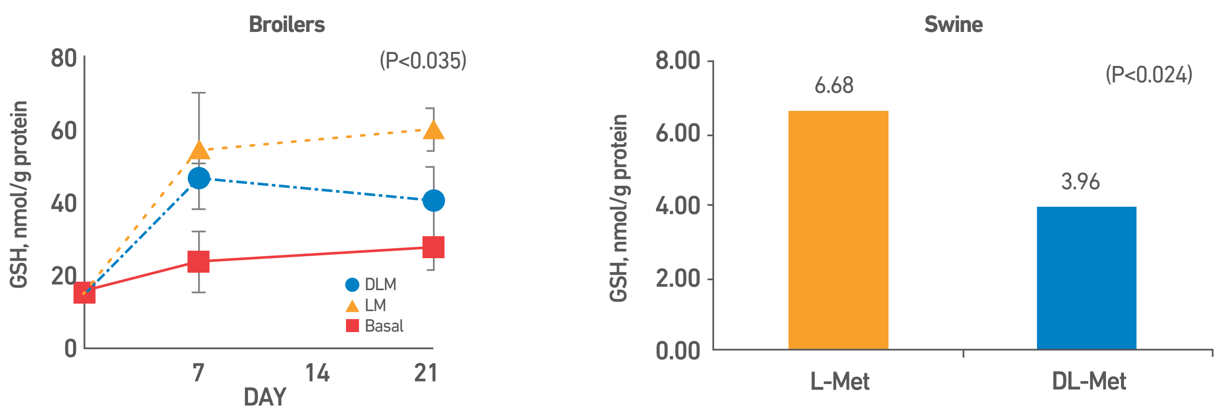

Figure 7. **Synthesis of glutathione (GSH) in the duodenum of young animals fed with two sources of methionine.**

Information from Shen et al. (2015):
. L-met: 0.56% of met from basal diet + 0.285% L-met
. DL-met: 0.56% of met from basal diet + 0.285% DL-met
. n = 11 per treatment.
. Analysis 7; 14 and 21 days of age
. Requirement 90% form Aviagen (2007).

Information from Shen et al. (2014)
. L-met: 0.16% of met from basal diet + 0.145% L-met
. DL-met: 0.16% of met from basal diet + 0.145% DL-met
. Requirement 95% of NRC (1998).
. n = 10 per treatment.
. Weaning = 21 days and initial weight = 8.40±0.25 kg.
. Experimental period = 20 days after weaning.

The functional effect of methionine in the gastrointestinal tract proves it antioxidant effect (better redox status or reduced oxidative stress), being key to healthy intestinal growth with consequent impact on animal performance, especially in young animals. L-methionine, is readily metabolized in the gastrointestinal tract for intestinal development with better GSH production, while D-methionine is not the biologically active form in the intestine.
In conclusion, L-methionine is the most appropriate methionine source for young animals, being the most efficient amino acid to serve intestinal cells and promote better redox status when compared to DL-methionine.

Under field conditions, it is possible to conclude that L-methionine is the best option among methionine sources in the intestinal health program, and synergistically support other additives on banning of antibiotics in poultry and swine feeds.
Methionine has another important biological factor in juvenile animals. For example, Stringhini et al. (2016) reported that enzyme secretion in chicks reaches its peak at four days of age for amylase and trypsin enzymes and it remains relatively constant for lipase up to 10 days of age. To prove the lower enzymatic activity given to young animals, D'Aniello et al. (1993) quantified a low amount of oxidase enzymes (responsible for the conversion of D-amino acid isomer to L-amino acid isomer) in young animals when compared to the adult phase as shown in Table 1 and Figure 9. The activity of oxidase enzyme is 50 to 150 times lower in the first days of life when compared to adults. This means that the saturation point or enzymatic activity is low, consequently, in a greater intake of D-amino acids, excessive accumulation will occur in the tissues, favoring the occurrence of physiological damage, such as; suppression in the synthesis of essential enzymes to the organism and growth inhibition. This evidence probably indicates one of the reasons for the greater toxicity of methionine compared to other amino acids, especially when it comes to young animals submitted to a low-protein diet with excess methionine (Benevenga et al., 1984; Harper et al., 1970).

Table 1. D-amino acid oxidase (DAAO) and its correlation with age in mouses

Mouses	D-AAO (milliunits/g wet tissues)		
	Liver	Kidney	Total
2 days of age	0.81	1.53	2.34
18 days of age	15.6	180.4	196.0
80 days of age	49.5	260.3	309.8

Adapted from D´Aniello et al. (1993).

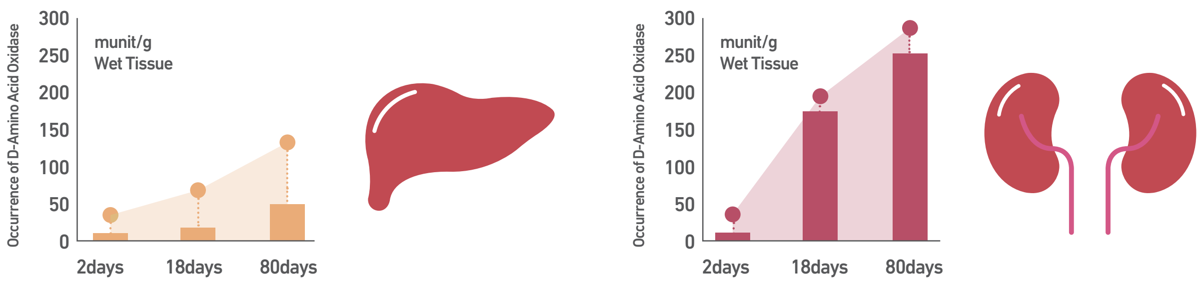

Figure 8. illustration of D-AAO in the liver and kidney according to D`Aniello et al. (1993).

Importance of L -methionine in adult animal nutrition

The starter phase of broilers and swine is a crucial point to reach maximum final performance. The performance losses in the starter phase will hardly be redeemed with compensatory growth during the growth and final phase. Given this fact, the best nutritional model for poultry and swine is based on the fact of offering adequate nutrient consumption, avoiding animal metabolism inefficiencies and field challenges as much as possible.

Unlike young animals, D'Aniello (1993) establishes that in adult animals, the ingestion of D-amino acids does not accumulate in the tissues due to the availability/enzymatic activity, which brings the feeling that the isomers become an irrelevant topic in this life stage.

On the other hand, recognizing that the conversion of the D to L isomer of amino acids, for the most part, occurs in the liver and kidneys (Saunderson, 1985), it is possible to affirm that hepatic efficiency is directly linked to the quality of the ingredients that make up a diet. Feeding with mycotoxins, peroxides, and high crude protein content will directly affect hepatic and renal metabolism, and probably affect the rate of amino acid isomerization.

Offering bioactive L-Methionine in the growth and finishing phase of poultry and swine, in addition to improving performance, is expected to become efficient in liver protection as well as efficient in the detoxification process of animals.

Marchioro et al. (2021), evaluating the protective effect of methionine for broilers when submitted to diets contaminated with aflatoxins, observed significant improvement in the performance of birds at 42 days of age (Table 2).

Table 2. **Performance and relative liver weight of those chickens intoxicated with aflatoxins, supplemented or not with methionine - 42 days of age.**

Treatments	Feed Intake, g	Weigh day-42	FGR	Relative Liver Weight, g
Control	4095^{a}	2405.09^{a}	1.70^{b}	2.54^{c}
Met 2x	3999^{a}	2426.08^{a}	1.65^{bc}	2.50^{c}
Met 3.5x	4043^{a}	2451.11^{a}	1.65^{c}	2.47^{c}
Afla	3574^{b}	1930.72^{c}	1.85^{a}	3.59^{b}
Afla+Met 2x	3226^{c}	1995.52^{b}	1.61^{bc}	3.9^{a}
Afla+Met 3.5x	3335^{c}	2110.75^{b}	$1.66b^{bc}$	3.95^{a}

DL-Metionina: 2,5 kg/MT.
Aflatoxina: 2,8 mg/kg.

The result shows the importance of methionine in the liver detoxification process. Thus, it is possible to state that increasing the methionine recommendation when there is an increase in mycotoxin contamination can be an important nutritional solution for poultry and swine.

In this article, we cannot forget to mention the bioequivalence of methionine sources. Research carried out by Esteves-Garcia (2018) was applied to the bioequivalence of DL-Methionine when compared with L-Methionine for weight gain and FGR of 89% and 77%, respectively.

Hannas et al. (2018) using piglets of 15 to 30 kg found bioequivalence of L-Methionine when compared to DL-Methionine of 105.49% and 134.30% for daily weight gain and feed efficiency, respectively.

These results indicate a biological superiority of L-Methionine over DL-Methionine. Our experience by field trials and scientific research leaves the perception of L-Methionine is 20% superior when compared with the DL-Methionine.

Reference

1. Burley, H.K., Anderson, K.E., Patterson, P.H. and Tillman, P.B. 2016. Formulation challenges of organic poultry diets with readily aailable ingredientes and limitd synthetic methionine. J. Appl. Poultry Res. V. 25, o. 443-454.
2. Chen, Y. Li D., Dai, Z., Piao, X., Wu, Z., Wong, B., Zhu, Y., Zeng, Z. 2014. L-Methionine supplementation maintains the integrity and barrier fiuntions of the small-intestinal mucosa in pat-weaning piglet. Amino acids nutrition.
3. D´Aniello, D´Onofrio, G., Pischetola, M., D´Aniello G., Vetere A., Petrucelli L., Fisher, G.H. (1993). Biological role of D-amino Acid Oxidase and Aspartate Oxidase. The Journal of Biological Chemistry, v. 268, n.36, p. 26491 – 26949, 1993.
4. Garlick, O. 2006. Toxicity of methionine in humans. American Society for Nutrition, p. 1722S-1725S.
5. Harper, A.C., Benevenga, N.J., Wohlhueter, R.M. 1970. Effects of ingestion disproportionate amounts of amino acids. Phisiologil Reviews, v. 50 n.8, p. 429-539.
6. Leeson, S. Summers, J.D. 2001. Scott´s Nutrition pf the Chicken, p. 591.
7. National Organic Standard Board (NOBS) Handling Subcommitee Petitioned Material Proposal L-Methionine, January, 2017. www.ams.usda.gov
8. Noy, Y., Sklan, D. 1998. Metabolic response to early nutrition. Journal Applied Poultry Research, v. 7, p. 4367-451.
9. Marchioro A., Dilkin P., Mallmann C.A., Mallmann A.O., Tyska D., Ceccantini M., Neves W.L., Giacominini L., Rauber, R.H. (2010). Eficiência da metionina na diminuição dos efeitos tóxicos das aflatoxinas em frangos de corte. IV Congresso Latino Americano de Nutrição Animal CBNA/AMENA. P. 31
10. Oliveira Neto A.R. 2014. Metabolismo e exigência de metionina. In: Nutrição de não ruminantes, Seção III – Proeteínas e Aminoácidos, p. 186 – 211.
11. Saunderson, L. (1985). Compartive metabolismo of L-methionine, DL-methionine and DL-2-hudrody 4- methyltiobutanoic acid by broiler chicks. British Journal of Nutrition, 54, p. 621-633.
12. Shen, Y.B., Weaver, A.C., Kim, S.W. 2014. Effect of feed grade L-methionine on grwth performance and gut health in nursery pigs compared with conventional DL-methionine. Journal animal Science, n.92, p. 5530-5539.
13. Shen, Y.B., Ferket, P., Park, I., Malheiros, .D., kim, S.W. 2015. Effects of feed grade L-methionina on intestinal redox status, intestinal development, and growth performance of young chickens compared with coonventional DL-methionine. Journal Animal Science, v. 93, p. 2977 – 2986. Doi:10.2527/jas 2017-8898.
14. Stringhini, J.H., Carvalho, F.B., Martins, P.C., Santos, J.S., Carvalho, D.P., Pires, M.F., Rezende, P. M., De Oliveira, E.M. 2016. A importância das dietas pré-iniciais para aves – parte 01. Avicultura Industrial, n. 10, ano 108, edição 1260.
15. Zhang, S., Gilbert, E.R., Noonan, K.J.T., Saremi, B., Wong, E.A. 2018. Gene expression. And activity of methionine converting enzymes in broiler chickens fed methionine isomers or precursors. Poultry Science, v. 97, p. 2053-2063.