# Response to Letter to the Editor titled: Issues with a meta-analysis assessing the efficacy of different sources of methionine supplementation by A. Lemme and H-P Piepho.

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We are responding to letter to the editor regarding our recent paper titled 'Evaluating growth response of broiler chickens fed diets supplemented with synthetic DL-methionine or DL-hydroxy methionine: a meta-analysis' by Uddin et al. (2022) Poult. Sci. 101:101762 https://doi.org/10.1016/j.psj.2022.101762.

## DATA SELECTION

The data selection strategy has been described in the materials and methods of the paper. The publication search process for this study has been as extensive and complete as possible. A total of 3,279 publications were identified through the literature search. Although the inclusion criteria were provided in the paper, these items are further clarified here. The papers were selected using the following inclusion criteria: a) articles that compared DL-Met and OH-Met where studies indicated the source and levels of the Met, b) articles with detailed description of diet composition, c) articles that reported at least 2 of these variables: feed intake, weight gain, or feed conversion ratio (**FCR**), d) articles without interactions other than the Met effects, and e) articles that were published in peer-reviewed journals in English, Portuguese, or Spanish. No screening regarding authors or affiliations occurred.

In addition to searching the online databases, we have compared the articles found in our search to the list of articles in Sauer et al. (2008). Thus, 4 papers were found missing from our initial search. Among these papers, 2 articles were included in the database, one paper was not found, and the other was classified as a research note. The authors of the letter to editor listed several publications as missing. Lemme et al. (2002) and Payne et al. (2006) had both been included in our database, as listed in the references (Appendix Ref. #15 and #21). As has been done for all papers screened, those reporting several experiments have been included as different trials with a unique identifier. Table 1 lists the reasons for inclusion or exclusion of papers listed in the letter to the editor.

As explained again above, the selection criteria were very clear, so we do not agree with authors' contention that 30% records were omitted. All the papers they listed were in the original studies' list and were screened according to the criteria we outlined. This search identified 3,279 articles initially. The inclusion criteria could be debated, but no deliberate exclusion of any studies occurred as the authors implied.

## NUTRIENT REQUIREMENTS

The objective of this study was to summarize the current literature available on Met sources for broiler chickens and to predict the weight gain response to Met intake using different mathematical growth functions. One hundred twenty-seven articles were excluded based on the reasons presented in Uddin et al. (2022), that is, 1) not in the scope, 2) only one source of Met, 3) duplicates, 4) conference abstract, 5) studies with challenges, 6) no performance data, 7) impossible to recalculate diet composition, 8) not standard broiler breeds. Several dose-response studies performed on only one Met source were excluded as our analysis did not seek to determine recommendations for Met and Met + Cys. The fitting was only done to determine the linear phase of the study, which is the most relevant to the meta-analysis. For our objective of comparing Met sources, the accuracy of the 'requirement' is immaterial. In this study, we fitted different



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**Table 1.** Reasons for the inclusion or exclusion of the articles listed by Lemme and Piepho in their letter to the editor.

| Reference | Inclusion | Comments |
|---|---|---|
| Hoehler D, Lemme A, Jensen SK, Vieira SL 2005. Relative effectiveness of methionine sources in diets for broiler chickens. J Appl Poult Res. 14:679-693 | yes | This article reported several studies comparing DL-Met, diluted DLM (65%) and liquid MHA-FA. Trial 1 was divided in two phases (1−21 d and 21−42 d); Trial 2 was divided in 3 phases (1−18 d, 19−28 d and 29−39 d); Trial 3 in three phases (1-21, 22−32 and 33−42 d); Trial 4 was run from 7 to 35 d and Trial 5 divided in two phases (7−21 d and 22−40 d). Several types of diets were offered in each phase of each trial.<br>This article was included as it matches the criteria of inclusion. Relationship between predetermined variables (i.e., ADG vs. animal age; ADG vs. feed intake, ADG vs. Met dose and ADG vs. Met intake) were visually assessed using scatter plots for each growth phases for visual identification of outliers.<br>From this examination resulted in the removal of records as the authors reported only the overall performance, thus making it impossible to match the Met and Met + Cys intake to the weight gain in each phase. |
| Lemme A, Hoehler D, Brennan JJ, Mannion PF 2002. Relative effectiveness of methionine hydroxy analog compared to DL-methionine in broiler chickens. Poult Sci 81:838-845 | yes | Included in the database as indicated Line 46 of the supplementary material (#15). |
| Payne RL, Lemme A, Seko H, et al. 2006. Bioavailability of methionine hydroxy analog-free acid relative to DL-methionine in broilers. Anim Sci J. 14(1):455-458 | yes | Included in the database as indicated Line 65 of the supplementary material (#21). |
| Balnave D, Oliva A. 1990. Responses of finishing broilers at high temperatures to dietary methionine source and supplementation levels. Aust J Agric Res. 41:557-564 | no | This study, performed under high temperature, was outside the scope. |
| Buresh RE, Harms RH. 1986. Comparison of DL-Methionine and Alimet with broilers when full fed or restricted. Nutr. Rep. Int. 33:449-457 | no | Full-length version of this article was not found in the databases searched. |
| De Groote 1990 Efficacy of the dimeric form of methionine hydroxy analogue free acid as determined by chick bioassay. Ann. Zootech. 39:45-51 | no | Full-length version of this article was not found in the databases searched. |
| Huyghebaert G. 1993. Comparison of Dl-methionine and methionine hydroxy analogue-free acid broilers by using multi-exponential regression models. Br Poult Sci. 34:351-359 | no | Only the regression models and graphs were reported. No data available to be entered in the database. |
| Romer and Abel. 1999. Effects of DL-methionine hydroxyanalogue (MHA) or DL-methionine (DL-Met) on N-retention in broiler chickens and pigs. Anim Feed Sci Technol. 81. 193-203 | no | The diet composition did not match the criteria of inclusion as it was composed of 40% of field beans. In addition to being scarcely used in practical poultry diets, it was not possible to recalculate the diet composition using the PPFR program. |
| Rostagno, H. S., Barbosa W. A. 1995. Biological efficacy and absorption of DL-methionine hydroxy analogue free acid compared to DL-methionine in chickens as affected by heat stress. Brit Poult Sci. 36:303-312 | no | This study, performed under heat stress conditions, was outside the scope. |
| Vanweerden E. J., Schutte J. B., Bertram H. L. 1992. Utilization of the polymers of methionine hydroxy analog free acid (MHA-FA) in broiler chicks. Arch Geflugelkunde 56:63-68 | no | The two experiments reported here, were designed to obtain more information about the utilization of the polymers of DL-MHA-FA in chicks. The diet composition did not match the criteria of inclusion as it contained 30% of Tapioca, which was classified as exotic ingredient, not used in practical diets. |
| Wallis IR. 1999 Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing broiler chickens. Aust J Exp Agric. 39:131-141 | no | Ross 1 broiler cockerels were used in this study. Discarded as one of the selection criteria is the use of standard growing breeds. |

models (linear-plateau, quadratic-plateau, and piecewise-linear model) to determine parameters of interest. Models were compared based on the leave-one-out cross-validation information criterion (**LOOIC**; Vehtari et al. (2017)). The LOOIC is one of the methods for estimating prediction accuracy for Bayesian models. which is advantageous over simpler estimates of predictive error such as AIC or DIC (Vehtari et al., 2017). Figure 3 and 4 in Uddin et al. (2022) clearly show the breakpoints ($\kappa$) which varied between models fitted for the dataset where LOOIC were used to select the best fitted models.

Data visualization and exploratory analysis indicated a nonlinear response of ADG to Met and Met + Cys intake. In the literature, some authors recommended a low bio-efficacy for OH-Met when Met + Cys are below requirements and an equivalent efficacy at the requirement (Agostini et al., 2016). Indeed, the efficiency of utilization of an amino acid follows a quadratic response to its intake. When an animal is fed above its requirement, low efficiency of utilization is observed because amino acid utilization changes according to the dietary level (Fatufe et al., 2004). This principle has been demonstrated for sulfur amino acids (Fatufe and Rodehutscord, 2005; Reis et al., 2018). Therefore, a cut-off point has been determined as the requirement of the groups of animals within our database. The efficacy of Met sources was assessed by only focusing on the range where animals respond the most to the supplementation and where there is most likely a chance to find a difference.

The cut-off point varies in each phase and depends on the model used (linear-plateau, quadratic-plateau, piecewise model) as presented in Figures 3 and 4 (Uddin et al., 2022). These figures show, the determined cut-off points are much higher with the quadratic-plateau model than with the linear-plateau model. In starter phase, 85% of the data supplemented with synthetic Met sources was retained in the linear range of the curve to dMet + Cys intake whereas in the grower and finisher feeding phases, the cut-off point obtained with the quadratic-plateau

model included most of the datapoints. We consider this to be enough for a fair comparison between Met sources. In addition, this highlights that most of the studies published on the comparison of Met sources did not always supply adequate dMet + Cys as most of the data is below the plateau. Figure 4 in Uddin et al. (2022) showed that only 4 datapoints were above the cut-off point determined with the quadratic-plateau model.

We summarized the dietary composition of the treatments included in the database in Table 1 (Uddin et al., 2022). Average levels, SD, min, and max levels were given for dLys and other essential amino acids. While this information was given to describe the database, the letter's authors calculated ratios of dMet + cys to dLys. Calculating a ratio of dMet and dMet + Cys to dLys based on the average value of dLys is misleading given the variability of the datapoints. The average dLys level in the starter phase is of 1.15%, whereas the respective min and the max are of 1.00 and 1.58%. This confirms the discrepancy in the database and reinforces the meticulous evaluation of the studies selected, as recommended by Sauvant et al. (2008). If the objective of this study was to provide a recommendation, the ratios would have been calculated in each treatment of each study and modeled against the weight gain. In addition, the average dLys level is far below Ross (2019) recommendations, which is 1.28% for the 0 to 10 d period. In reaching this study's objective using a thorough methodology, we have concluded that no significant difference was detected in ADG in response to synthetic methionine forms (i.e., DL-Met and OH-Met) at or below the estimated requirement.

As explained by Sauvant et al. (2008) (this reference is not in the reference list), "*...there are at least three steps necessary to effective data filtering in a meta-analysis. First, the analyst must ensure that the study under consideration is coherent with the objectives of the meta-analysis. That is, the meta-analytic objectives dictate that some traits must be measured and reported. The second step consists of a thorough and critical review of each publication under consideration, focusing on the detection of errors in the reporting of quantitative results. This underlines the importance of having a highly trained professional involved in this phase of the study. Only after publications have passed this 'expert' quality filter should their results be entered in the database. In this third step, it is important to ensure that a selected publication does not appear to be an outlier with respect to the characteristics and relations under consideration*". The clear goal was to quantitatively assess the efficacy of synthetic Met sources and determine differences in growth rate of broilers fed at or below requirements in response to Met intake. Using powerful Bayesian meta-analysis approach including the most recent studies, we have shown that no significant statistical difference was detected in ADG in response to the most common dietary synthetic methionine forms (i.e., DL-Met and OH-Met). Readers may reach their own conclusion with the analysis provided.

## STATISTICAL ANALYSIS

Between-study heterogeneity is a common phenomenon in meta-analysis. To address between-study heterogeneity in agreement with the letter to the editor, we fitted random effects of study which is customary and appropriate in meta-analysis. In meta-analysis, within-study covariances due to dependency is most common. Addressing within-study covariance using multivariate meta-analysis over univariate method would improve precision of analysis in some, but not all cases (Wei and Higgins, 2013). We set all off-diagonal elements of covariance matrix ($\Psi$) to zero (0) indicating independent study effects which is a common assumption in meta-analysis.

## DISCLOSURES

The authors MEU and EK stated no conflict of interest. The authors DIB and FR are affiliated with the funding agency.

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