Issues with a meta-analysis assessing the efficacy of different sources of methionine supplementation

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2022 Poultry Science 00:102115
https://doi.org/10.1016/j.psj.2022.102115

The article “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) analyzed literature data on methionine supply for broilers to derive requirement figures as well as to compare two methionine sources. We have concerns regarding both the reported results and the data analysis methodology. First, the data compilation is incomplete to achieve the objectives of the paper including determination of requirements and comparison of methionine supplement efficiencies. Second, the paper suggests Met and Met+Cys requirements of broilers but makes no attempt to discuss them in the context of nutrition of modern broiler strains. Third, the data preparation for methionine source comparison as well as the mathematical approach includes weaknesses resulting in misleading conclusions.

Selected data were incomplete: The authors analyzed 480 records from 39 studies comparing DL-Methionine (**DL-Met**) and DL-Hydroxy analogue of methionine (**DL-OH-Met**) in simultaneous dose-response experiments with broilers. While the supplementary material suggests 39 publications, more than 39 studies were available as for example, Lemme et al. (2002) and Payne et al. (2006) reported 2 and 3 studies, respectively. While the reported studies were found according to the process described, the authors also referenced another meta-analysis (Sauer et al., 2008) on the same subject. The second meta-analysis referenced at least 16 additional studies which were not considered by Uddin et al. (2022), providing 144 records from nine peer-reviewed papers (Buresh und Harms, 1986; Balnave und Oliva, 1990; Groote et al., 1990; van Weerden et al., 1992; Huyghebaert, 1993; Rostagno und Barbosa, 1995; Roemer und Abel, 1999; Wallis, 1999; Hoehler et al., 2005). All records meet the requirements as defined (Uddin et al., 2022) meaning that at least 30% records for analysis were missing. Interestingly, most of the missing studies reported DL-Met to be advantageous over DL-OH-Met with respect to biological effectiveness. Moreover, the selected studies were used for the determination of Met and Met + Cys requirements. Valuable research from 100 papers reporting studies with only one methionine source were excluded. This significant data omission appears deliberate but the rationale for this exclusion was not given. The power, outcome, and conclusions of the meta-analysis with respect to requirements would change when including such studies. We see no reason for excluding studies with just a single methionine source, because meta-analysis allows integrating such information with studies comparing both sources (Salanti et al., 2010). This is particularly relevant when the studies have a control, the usual situation, because the controls serve as a common reference to connect studies with just a single methionine source. Even without a control, the random-coefficient approach taken by the authors would have allowed including such studies, making use of inter-study information (van Houwelingen et al., 2002).

Reported requirement figures are questionable: Uddin et al. (2022) reported digestible Met+Cys requirements of 0.314 (LP; linear-plateau) or 0.379 g/d (QP, quadratic plateau), 0.932 g/d (LP), and 0.953 g/d (LP) for starter (11 days of age), grower (21 days of age) and finisher broilers (35 days of age), respectively. No statistical information on goodness of fit is provided except for the LOOIC which is only used to compare models but gives no easily interpretable absolute indication of fit to data. Figures 3 and 4 do not suggest a particularly suitable fit for the regression models, nor would they suggest break

DOI of original article: http://dx.doi.org/10.1016/j.psj.2022.101762
Received May 13, 2022.
Accepted August 2, 2022.

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points. In addition, it appears dubious to include data from both DL-Met and DL-OH-Met responses at the same time. Despite of the author's conclusion that the effectiveness of these two methionine sources is the same, there is scientifically justified reason to assume that biological efficiency of these methionine sources differs (Jansman et al., 2003; Sauer et al., 2008; Lemme et al., 2020). In contrast to the reported Met + Cys requirements, Rostagno et al. (2017) suggest requirements of 0.47 or 0.57, 0.88 or 1.12 and 1.24 or 1.66 g digestible Met + Cys/d for 11, 21, and 35 day old low performing female broilers or high yielding male broilers, respectively. Accordingly, requirements proposed by Uddin et al. (2022) are very low and would not agree with commercially established specifications either. Moreover, relating the digestible Met+Cys requirements to the reported average digestible lysine levels (Uddin et al., 2022) would reveal ratios of 64 (LP) or 77 (QP), 83 (LP), and 65% (LP) for starter, grower and finisher broilers, respectively. These numbers are well below 74%, which is proposed to be ideal (Rostagno et al., 2017; Spek, 2018) especially for starter (LP) as well as the finisher phase. Linear-plateau regression results in lowest requirement estimates compared to nonlinear regression approaches (Rodehutscord und Pack, 1999), the reported requirements are not applicable for broiler nutrition to avoid tremendous impairments in growth, meat deposition, and feed utilization.

Comparison of methionine sources: In principle, the authors applied a slope-ratio assay for 3 growth phases. All data points above the determined requirements (LP or QP) were excluded from analysis without considering whether individual studies might have suggested requirements above or below these averages as demonstrated by Morris (2004). Visualization and analysis according to mixed model procedures (St-Pierre, 2001) would have recognized this difference. In addition, the requirement determination data were regressed against Met or Met + Cys intake. Thus, a nutritional value for DL-OH-Met was already assumed before it was determined which appears to be a circular reference error affecting the final outcome both for requirements and comparison of supplements. As explained by Uddin et al. (2022), for methionine source comparison the digestible Met (and Cys?) intake of basal treatments was subtracted from the overall intake per treatment, yielding the intake of methionine supplements. For the subset Met + Cys, Cys would therefore just be a constant. Indeed, the determined requirement figures for digestible Met or Met+Cys would decide which data points were considered or excluded. Still, all responses above basal treatment would be assigned exclusively to product intakes but not to Cys intake. Therefore, the evaluation on basis of Met+Cys a priori reduced the relative differences of intake increments and, therefore, prevents a fair differentiation between supplements. The authors further duplicated records for basal treatments with no Met source supplementation, which would invalidate the joint analysis comprising both supplements. Such duplication is unnecessary because the same control value can be used in different regressions. The authors stated that recent empirical studies and meta-analyses on methionine sources were lacking. However, Lemme et al. (2020) published a comprehensive paper on methionine source comparison well before submission of the current work. The paper included a validation of the methodology to determine the relative biological effectiveness of methionine sources besides providing evidence that DL-OH-Met was 65 to 74% as efficient than DL-Met (molar comparison). Interestingly, comparing slopes from starter and grower (Met, LP) approaches by Uddin et al. (2022) would suggest DL-OH-Met slopes 85% and 79% as steep as the DL-Met slopes. Although these slopes did not differ significantly from DL-Met slopes, their numbers are confirming those suggested by Sauer et al. (2008). We would like to add that a nonsignificant difference between supplementation sources does not prove that there are no differences, or in the words of Altman und Bland (1995): "Absence of evidence is not evidence of absence." Insufficient data make nonsignificant results more likely, even if there is a relevant effect.

Moreover, several published experiments challenging and validating a biological equivalency of 65% for DL-OH-Met (equivalent to 73% on molar basis) compared to DL-Met (100%) in poultry should have been considered in the discussion (Hoehler et al., 2005; Payne et al., 2006; Santos Viana et al., 2009; Agostini et al., 2017; Lingens et al., 2021).

Diagonal variance-covariance matrix for heterogeneity is unrealistic: The authors fit random effects for heterogeneity between studies as is customary and appropriate in meta-analysis. In the case at hand, the analysis involves regressions, turning the model into a random coefficients regression. It is crucial for such models to allow for a covariance between random intercepts and regression coefficients (Longford, 1994; van Houwelingen et al., 2002). Also, such covariances are generally to be expected and can be substantial (Sauer et al., 2008). Hence the diagonal variance-covariance matrix fitted by the authors, which assumes independence between coefficients, is problematic.

ACKNOWLEDGMENTS

Support of H.-P. Piepho by the German Research Foundation (DFG grant PI 377/24-1) is appreciated and acknowledged.

DISCLOSURES

The authors have no conflicts of interest to report.

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