# LETTER TO THE EDITOR

# Noninferiority of the hydroxy analog of methionine compared to DL-methionine not confirmed in a broiler trial

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Concerning: "New statistical approach shows that hydroxy-methionine is non-inferior to DL-methionine in 35-day old broiler chickens" by D. I. Batonon-Alavo, C. Manceaux, J. T. Wittes, F. Rouffineau, and Y. Mercier.

In the paper by Batonon-Alavo et al. (2023) a noninferiority test was used to provide evidence that broiler performance achieved by supplementing feed with DL-hydroxy-methionine (**OH-Met**) is not worse than that achieved with DL-methionine (**DL-Met**). The purpose of this letter is to point out that the noninferiority test was not applied correctly, and therefore the respective conclusions are not correct, and the paper ignores fundamental nutritional principles.

The authors do not explain in detail the process for selecting broiler studies for the meta-analysis required for the determination of the lower confidence limit according to the 95-95 approach suggested by Schumi and Wittes (2011). It appears that feeding studies with final age of 35 d and feeding corn-wheat-soybean meal based were selected. Studies testing liquid OH-Met or OH-Met-Ca in comparison to DL-Met were included but criteria for selecting particular treatments out of the dose-response trial remain unclear. While referring to Lemme et al. (2020) in the discussion, data reported in that paper were not considered for this exercise although in that study corn-wheat-soybean meal-based diets were fed until 35 d of age. Accordingly, a response of 963 g body weight gain (1.2 g/kg DL-Met vs. unsupplemented basal diet) was reported. Adding this to the meta-analysis would have even increased $M_1$ and, thus, increased the range of acceptable difference.

It turns out that Batonon-Alavo et al. (2023) have applied the 95-95 method described in Schumi and Wittes (2011) incorrectly. Here, we will briefly state the 95-95 method as proposed by Schumi and Wittes (2011) and also refer back to the original proposal of the 95-95 method given in Rothmann et al. (2003) and Rothmann and Tsou (2003). The method proposed first uses a meta-analysis to estimate the effect of the reference treatment compared to the control treatment. This effect is estimated as the difference between the reference treatment mean and the control treatment mean. The estimate will be referred to as $D_1$ (our notation). A conservative estimate of the effect that is likely to be observed in a new study is obtained by computing the 1-sided lower limit of a 95% confidence interval of that difference (Rothmann et al., 2003). The lower limit is denoted as $M_1$. The user then determines a predetermined lower fraction of $M_1$ that corresponds to the largest loss of effect, that is, the largest inferiority the user is prepared to consider acceptable. That smaller value is denoted as $M_2$. For example, if a reduction of the effect by 5% of $M_1$ is deemed acceptable, the value is $M_2 = 0.05{*}M_1$. $M_2$ is denoted as the noninferiority margin (Rothmann et al., 2003). To establish noninferiority of a new treatment compared to the reference treatment, it then needs to be shown that the difference of the new treatment to the reference treatment mean is significantly larger than $M_2$. We denote the estimate of this difference as $D_2$. Note that $D_2$ is expected to be negative, assuming that the reference treatment is superior to the new treatment. The difference $D_2$ corresponds to the estimated loss of effect when using the new treatment instead of the reference treatment. The decision is based on the lower 1-sided 95% confidence interval for the difference $D_2$ (Rothmann et al., 2003).

Batonon-Alavo et al. (2023; Table 1) find $M_1 = 467$ for body weight based on the difference of DL-Met compared to a basal diet. This calculation is correct, apart from the fact that the authors used a 2-sided 95% interval in place of a 1-sided 95% interval prescribed by Rothmann et al. (2003). The calculation of the noninferiority margin ($M_2$), however, seems to be in error. According



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to the footnote in their Table 4, the authors compute this as the difference between the lower limit $M_1 = 467$ and the mean difference $D_1 = 657$ in their Table 1 and find a value of $M_1$-$D_1 = -191$ for body weight. This value is used as the noninferiority margin and hence is compared to the confidence interval for the difference of OH-Met and DL-Met in their Table 4 to assess noninferiority. However, the way this value of −191 was computed is not in agreement with the way the noninferiority margin $M_2$ is computed according to Schumi and Wittes (2011; see description above), so the noninferiority test is done incorrectly. Clearly, |M1-D1| is the half-width of the confidence interval around D1. This only indicates how accurately the DL-Met vs. basal diet difference has been estimated. There is no way in which this measure of precision can inform the choice of the noninferiority margin.

We also note that the description of the calculations of $M_2$ from $M_1$ in Batonon-Alavo et al. (2023) is in error. The authors assert that if the acceptable loss is 100%, then $M_2 = 0$, and that with an acceptable loss of 0%, the choice is $M_1 = M_2$. But the complete opposite is true! The authors say that they computed a noninferiority margin $-\delta_L$ (erroneously computed by the authors as $M_1$-$D_1$) assuming no loss of efficiency for OH-Met compared to DL-Met. There are 2 problems here: 1) It is unclear why the alternative notation $-\delta_L$ is introduced for the noninferiority margin, which really should correspond to $M_2$. 2) When assuming an acceptable loss of 0%, then $M_2 = 0$. This clearly would mean that no loss of efficiency at all is accepted. To establish that there is no loss, one would actually need to show that the "new treatment" OH-Met is significantly superior to DL-Met. But this is not what the authors have done.

Based on an experiment with 35 replicates per treatment with 40 broilers per replicate, Batonon-Alavo et al. (2023) reported a 23.2 g lower body weight for OH-Met fed birds compared to DL-Met fed birds in their feeding experiment. The respective lower CL for the difference between OH-Met and DL-Met (−50.8 g) was higher than their falsely computed the noninferiority limit of −191 g ($M_1$-$D_1$) and, consequently, noninferiority was concluded. Actually, the authors stated that even a 20% loss of efficiency "for amino acids is unacceptable on a practical point of view" (Batonon-Alavo et al., 2023). Therefore, they suggested an acceptable loss of efficiency of 3 to 5% which would mean correctly computed $M_2 = 0.03 * M_1 = 0.03 * (-467\text{ g}) = -14.0$ g to $M_2 = 0.05 * (-467\text{ g}) = -23.4$ g acceptable difference in body weight gain of OH-Met fed birds compared to DL-Met fed birds. Therefore, the mean difference in body weight (−23.2 g) of the OH-Met treatment and particularly the lower CL (−50.8 g) were not above $M_2$ and, thus, noninferiority cannot be confirmed!

The authors concluded "that OH-Met was noninferior to DL-Met" and "that the two Met sources similarly sustained growth performance" and, therefore, confirm other studies on effectiveness of OH-Met in comparison to DL-Met. This conclusion is misleading as the experimental set-up of the reported trial is not suitable to assess effectiveness of OH-Met relative to DL-Met. The chosen digestible methionine+cysteine levels in the experimental diets were according to breeder recommendations while also treatments chosen for the determination of $M_1$ were at requirement or asymptotic level, respectively. In response to Vázquez-Añón et al. (2006) (a study which was used in the meta-analysis of the present paper), Hoehler (2006) demonstrated that comparisons between OH-Met and DL-Met at levels of commercial use which means at asymptotic performance level result in "unsound and misleading" conclusions. Like Lemme et al. (2020), he demonstrated that pairwise comparisons of OH-Met and DL-Met treatments would reveal substantial performance differences at low supplementation which are diminishing with increasing supplementation levels. This is related to changing marginal utilization toward meeting broiler requirements following the law of diminishing returns. The trial data but also a meta-analysis by Lemme et al. (2020) demonstrated that even diluted DL-Met with a purity of 65%, which was included in the trial as internal standard, would allow for same performance as with pure DL-Met once supplemented high enough. This latter paper suggested a relative bioefficiency of 63% for OH-Met compared to DL-Met on product (as is) basis which means that at any supplementation level and at any dietary Met+Cys level 630 g DL-Met are equivalent to 1,000 g OH-Met. There are several examples published demonstrating that a replacement of OH-Met by DL-Met at a ratio of 100:65 on weight basis reveals similar, if not identical, broiler performance (Mandal et al., 2004; Payne et al., 2006; Yao et al., 2006; Santos Viana et al., 2009; Pagliari Sangali et al., 2014). Interestingly, the papers by Drażbo et al. (2015) and Zelenka et al. (2013), which were used for determination of $M_1$ by Batonon-Alavo et al. (2023), reported average relative bioefficiencies of 46% for OH-Met calcium salt and 62% for liquid OH-Met compared to DL-Met on product (as is) basis. Therefore, the supplementation levels and dietary methionine+cysteine levels chosen in the current publication are not suitable for assessing relative bioefficiency and noninferiority of OH-Met.

## DISCLOSURES

Andreas Lemme reports a relationship with Evonik Operations GmbH that includes: employment.

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