META-ANALYSIS

IMPORTANCE OF MEASURING RELATIVE BIOAVAILABILITY IN METHIONINE SOURCES

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Methionine (Met) is an essential amino acid of particular importance because it cannot be synthesized by broilers and because it can be converted to the other sulfur amino acid, cysteine (Cys). Due to low levels in ingredients, Met and Cys are usually the first performance limiting amino acids in broiler feeds and commonly DL-methionine (**DL-Met**, 99% purity) or methionine hydroxy analog (**MHA**; free acid: MHA-FA, 88% purity; to a much smaller extent calcium salt: MHA-Ca; 84% purity) is supplemented to meet requirements. Due to the chemical differences between the two compounds, their nutritional values differ. Nutritional value is expressed as the relative bioavailability (**RBV**) of MHA compared to DL-Met, which indicates the replacement ratio of these products in feed.

A recent publication indicated an RBV at or close to 65% for MHA-FA compared to DL-Met in broilers on a product-to-product basis [1], and a scientific opinion published in 2018 in the EFSA journal concluded an RBV of 75% on an equimolar basis for both forms of MHA (64 (MHA-Ca) - 67% (MHA-FA) on a product basis) based on the available literature at that time [2]. The principle behind the RBV determination is to compare dose-response data for both products simultaneously [3]. In this approach, the starting point (basal diet) of the curve and the asymptote are the same for both products while the difference between the slopes (regression coefficient) of the curves is used to calculate the RBV.

The multiple exponential regression method was validated in a meta-analysis, which provided statistical evidence that both methionine sources would allow for the same maximal performance (asymptote), while the steepness of the curve indicates their nutritional value [4]. Earlier experiments [5–8] provided additional evidence for the appropriateness of the simultaneous dose-response approach by introducing diluted DL-Met (diluted to a purity of 65%; **DLM65**) as an internal standard. In a recent meta-analysis, Lemme et al. [1] found that DLM65 was 62% as efficient as DL-Met on average across six experiments, while MHA-FA was 63% as efficient. This result validates the methodology because the RBV of DLM65 was almost exactly as expected for DL-Met diluted to 65% purity. The RBV value in the cited studies is less than 88%, which is contrary to claims made by MHA producers and may have implications not only for animal performance but also for economics and purchasing decisions.

Based on this, an RBV of 65% is recommended for MHA products relative to DL-Met. Indeed, this finding should be reflected in the pricing of products to realize the full value. A recent challenge test at a commercial farm with 408,500 broilers suggested savings of >11,000 €/year just by applying our recommendation to a feed volume of 10,000 t/year [9].

The validation of the recommendation on relative bioavailability value

In addition to the dose-response studies, there is a more practical and simple experimental protocol that can be applied to challenge and validate the recommended RBV of 65% for MHA as compared with DL-Met (on product basis). The simplest test comprises two treatments with either MHA or DL-Met supplemented up to 65% as MHA, i.e. considering that 100 units of MHA are replaced by 65 units of DLM. The same animal performance at a lower cost is expected with DL-Met.

The application of such a performance test is exemplified by a recent publication, where both products were compared at different dietary Met+Cys levels under Northern European and Middle Eastern conditions [10]. The first study was conducted in Finland and consisted of 5 treatments with 9 replicates with 16 male Ross 308 broilers per replicate. Diets were wheat-soybean meal based. The second study was conducted in Jordan and consisted of 5 treatments with 10 replicates and 50 mixed-sex (1:1) Ross 308 broilers per replicate. In this case, corn-soybean based feeds were fed.

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In both trials, broilers received a basal diet (**BD**) formulated to meet all nutritional requirements except for Met+Cys (60-66% of Met+Cys requirements), or BD supplemented with MHA-FA to meet either 75% or 100% of Met+Cys requirements. In two further treatments, MHA-FA was replaced by DL-Met on a weight basis, but only up to 65% of the MHA-FA inclusion level, according to the recommended RBV of 65% for MHA-FA relative to DL-Met. Broilers were fed *ad libitum* from 0-35 days (Trial 1) or from 0-32 days (Trial 2) in 3-phase programs under standard housing conditions. The final results from trials 1 and 2 are presented in **Tables 1 and 2**, respectively.

Table 1.
Growth performance of male Ross 308 broilers fed with adequate and reduced Met+Cys levels and supplemented with either DL-Methionine (DL-Met) or liquid methionine hydroxy analogue free acid (MHA-FA) at a ratio of 65:100 during 35 days period

Performance parameters	**Basal**	**At reduced Met level (75%)**		**At recommended Met Level (100%)**		**SEM**	***P-value***
		100 MHA-FA	**65 DL-Met**	**100 MHA-FA**	**65 DL-Met**		
ADFI, g/d	62.19[a]	110.04[b]	108.53[b]	109.25[c]	110.46[b]	0.923	<0.01
ADG, g/d	35.85[a]	74.50[b]	73.94[b]	77.56[c]	77.95[c]	0.660	<0.01
FCR, g/g	1.735[a]	1.477[b]	1.468[b]	1.409[c]	1.417[c]	0.012	<0.01
BW, g	1260[a]	2574[b]	2555[b]	2678[c]	2691[c]	22.51	<0.01

ADFI=average daily feed intake, ADG=average daily gain, FCR=feed conversion ratio.
Data was analyzed using one-way ANOVA with GLM procedure of SAS (ver. 9.4). Significances were considered if P<0.05 (Tukey test).

Table 2.
Growth performance of mixed Ross 308 male and female broilers fed with adequate and reduced Met+Cys levels and supplemented with either DL-Methionine (DL-Met) or liquid methionine hydroxy analogue free acid (MHA-FA) at a ratio of 65:100 during 32 days period

Performance parameters	**Basal**	**At reduced Met level (75%)**		**At recommended Met Level (100%)**		**SEM**	***P-value***
		100 MHA-FA	**65 DL-Met**	**100 MHA-FA**	**65 DL-Met**		
FI, g	2592[b]	2879[a]	2855[a]	2867[a]	2933[a]	22.69	<0.05
BW, g	1586[c]	1914[b]	1916[b]	1980[a]	2014[a]	16.17	<0.05
WG, g	1545[c]	1873[b]	1874[b]	1938[a]	1972[a]	16.09	<0.05
FCR, g/g	1.673[a]	1.524[b]	1.510[bc]	1.470[c]	1.480[bc]	0.012	<0.05
CY, % of BW	70.75[b]	72.34[ab]	72.69[ab]	73.67[a]	72.88[a]	0.508	<0.05
BY, % of carcass	35.89[c]	38.75[b]	39.07[b]	41.60[a]	42.12[a]	0.417	<0.05

FI=feed intake, BW=body weight, WG=weight gain, FCR=feed conversion ratio, CY= carcass yield, BY= breast meat yield.
**Data was analyzed using one-way ANOVA with GLM procedure of SAS (ver. 9.4). Significances were considered if P<0.05 (Tukey test).*

In both studies, average feed intake was significantly lower in broilers fed the basal diet (indicated by different superscripts [a,b,c]) but there were no differences between the other treatments. In contrast, marginal dietary Met+Cys supply (75%) resulted in significantly lower weight gain and final body weight than treatments at 100% Met+Cys. These effects were reflected in feed conversion ratio, particularly, in trial 1 and breast meat yield in trial 2. However, there were no differences between the corresponding MHA-FA and DL-Met treatments at marginal or adequate Met+Cys supply, or between different performance parameters. This provides evidence that the recommended RBV of 65% for MHA-FA relative to DL-Met is applicable without compromising performance. Moreover, the observed lower performance at 75% of the recommended Met+Cys levels indicates a performance limitation by Met+Cys, which makes the 65:100 test more sensitive, and thus the results provide strong support for the recommendation.

Compilation and Meta-Analysis of previous preformance tests

While the above reports of the most recent trials challenge and confirm our recommendation, many such 65:100 trials have been conducted in recent years that can be analyzed by meta-analysis. For this compilation, we used the classical meta-analysis by using Hedges' g to estimate the effect size with a 95% confidence interval. This methodology is well established in animal science and has been used to integrate and determine the overall effect from several studies to provide more accurate insights [11]. Therefore, we conducted a meta-analysis to assess the performance responses of broilers to DL-Met when replacing MHA at a 65:100 ratio. Moreover, especially scientists and authors who favor – in contrast to us - a high nutritional value of MHA-FA, repeatedly state that the nutritional value of MHA-FA is higher, especially at or above Met+Cys requirement, while lower dietary Met+-Cys levels would interfere with the efficiency of MHA [12,13]. Therefore, we split the current meta-analysis of the 65:100 trials into experiments operating at marginal Met+-Cys supply (below recommendations), at recommended levels, and clearly above recommended levels to see if any differences in responses could be observed.

In order to perform the meta-analysis, the mean values of the performance criteria, the respective standard deviations and sample sizes (replicates per treatment) were extracted from each included study. The target variable reported in this article is the feed conversion ratio (**FCR**), but results for other performance parameters were almost identical. When more than one Met+Cys level was used in a study, each corresponding treatment pair (65:100) was coded individually. Additionally, the study groups were separated according to the Met+Cys level and classified in relation to the requirement (below, at or above requirement). In the case of dose-response studies, the requirement was determined by the exponential equation presented in the publication.

Data analysis was performed using Meta-Essential version 1.4. The estimated effect size (the difference between DL-Met and MHA treatment) was quantified using Hedges' g with a 95% confidence interval (CI) [14]. Data were pooled using a fixed-effect model due to the lack of heterogeneity, after being pre-checked using the I^2 statistic. An effect was declared significant when the overall estimated effect size was $P < 0.05$.

As shown in Figures 1, 2 and 3 no substantial heterogeneity was found for feed conversion ($I^2 = 0.00\%$), indicating that all studies in these subgroups produced an estimate of the same true effect size in a homogeneous population. In fact, the graphs demonstrate that all mean effect size values were close to zero and no single experiment had confidence intervals excluding zero, indicating very homogenous data with no exception. Moreover, with respect to the overall results indicated by the purple dots (and results shown in the last line in bold), the mean effect size was almost zero and the overall confidence interval was very small, providing clear evidence that replacement of MHA with DL-Met at a ratio of 100:65 always results in the same performance.

Study name	Hedges's g (95% CI)
Elwert et al. (2008)	-0.06 (-1.55 to 1.42)
Hoehler et al. (2005)	-0.07 (-1.56 to 1.41)
Hoehler et al. (2005)	0.18 (-1.58 to 1.94)
Hoehler et al. (2005)	1.02 (-0.85 to 2.88)
Lemme et al. (2020)	1.11 (-0.48 to 2.71)
Lemme et al. (2002)	1.25 (-0.67 to 3.17)
Payne et al (2006)	0.08 (-1.11 to 1.26)
Sangali et al. (2014)	-0.64 (-2.44 to 1.16)
Goes et al. (2017)	0.53 (-0.57 to 1.64)
Purohit et al. (2018)	-0.82 (-2.36 to 0.73)
Purohit et al. (2018)	0.19 (-1.30 to 1.68)
Purohit et al. (2018)	-0.30 (-1.80 to 1.19)
Purohit et al. (2018)	0.08 (-1.40 to 1.57)
Purohit et al. (2018)	0.75 (-0.79 to 2.28)
Purohit et al. (2018)	0.50 (-1.01 to 2.00)
Murakami et al (2017)	0.09 (-1.21 to 1.40)
Murakami et al (2017)	-0.66 (-2.00 to 0.68)
Murakami et al (2017)	-0.50 (-1.83 to 0.82)
Murakami et al (2017)	-0.18 (-1.49 to 1.13)
Overall (P = 0.488)	**0.10 (-0.17 to 0.38)**
Heterogeneity (I^2=0.00%)	

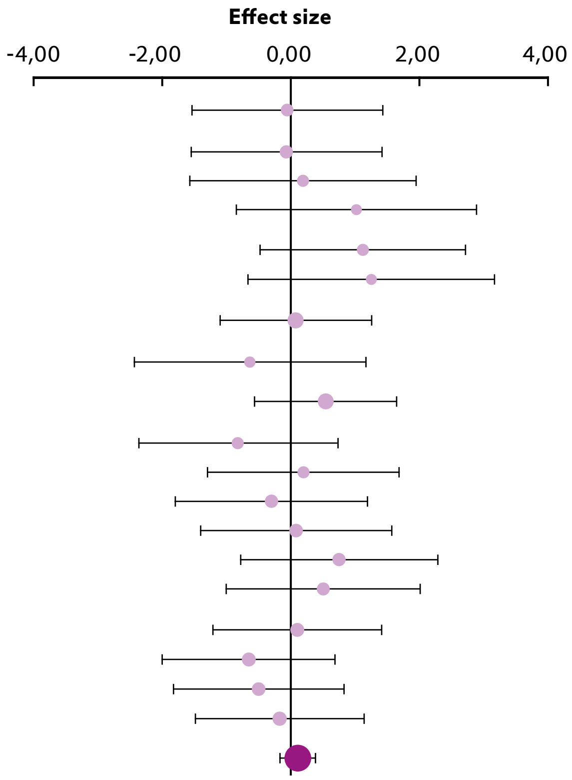

Figure 1. Forest plot showing the effect of dietary replacement of 100 parts MHA with 65 parts of DL-Met on feed conversion ratio of broilers when dietary Met+Cys is above the requirement.

Study name	Hedges'g (95% CI)
Lemme et al. (2020)	0.03 (-1.46 to 1.51)
Lemme et al. (2020)	-0.37 (-1.87 to 1.13)
Payne et al (2006)	-0.11 (-1.30 to 1.07)
Li et al. (2019)	0.22 (-0.87 to 1.31)
Li et al. (2019)	0.26 (-0.75 to 1.28)
Viana et al. (2009)	0.08 (-1.11 to 1.26)
Goes et al. (2017)	-0.07 (-1.16 to 1.01)
Boontarue et al. (2023)	-0.68 (-1.46 to 0.09)
Boontarue et al. (2023)	0.07 (-0.69 to 0.82)
Purohit et al. (2018)	0.60 (-0.92 to 2.12)
Purohit et al. (2018)	0.19 (-1.30 to 1.68)
Purohit et al. (2018)	-0.09 (-1.57 to 1.40)
Purohit et al. (2018)	0.92 (-0.64 to 2.48)
Purohit et al. (2018)	-0.95 (-2.51 to 0.62)
Purohit et al. (2018)	-1.18 (-2.79 to 0.43)
Lemme (2022)	-0.23 (-1.99 to 1.53)
Murakami et al (2017)	-0.44 (-1.76 to 0.88)
Murakami et al (2017)	0.05 (-1.26 to 1.36)
Fact&Figures n°15166	0.41 (-1.09 to 1.91)
Fact&Figures n°15133	0.15 (-1.61 to 1.91)
Fact&Figures n°15123	0.26 (-1.23 to 1.75)
Fact&Figures n°15120	-0.52 (-2.03 to 0.99)
Fact&Figures n°15119	-0.95 (-2.02 to 0.12)
Fact&Figures n°15116	-0.35 (-1.55 to 0.84)
Overall (P = 0.676)	**-0.13 (-0.33 to 0.07)**
Heterogeneity (I^2=0.00%)	

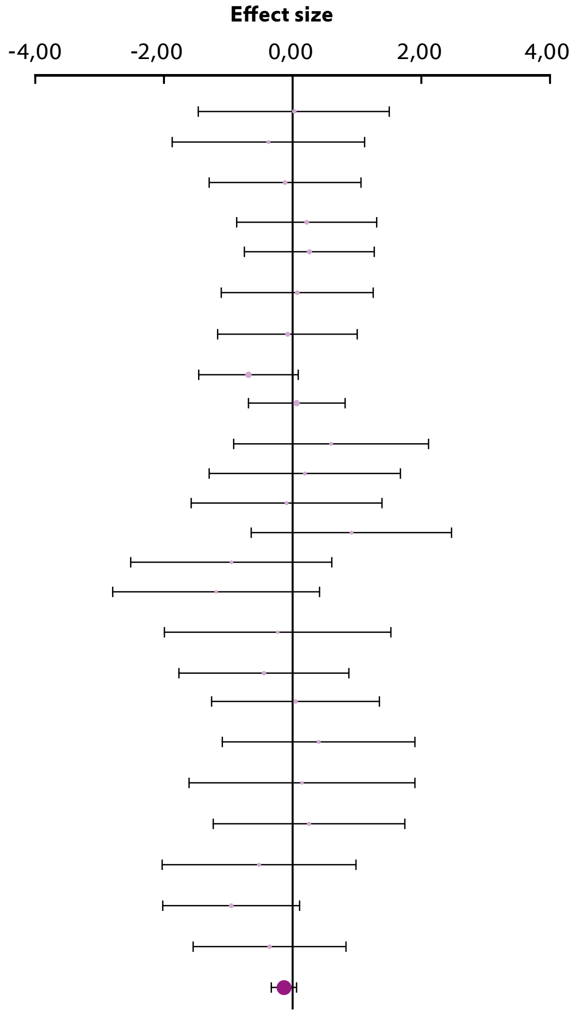

Figure 2. Forest plot showing the effect of dietary replacement of 100 parts MHA with 65 parts of DL-Met on feed conversion ratio of broilers when dietary Met+Cys is at the requirement.

In addition, analyzing all trials reported in Figures 1,2 and 3 together (not shown) also confirms that application of the recommended RBV of 65% for MHA-FA is always successful. Splitting, however, the data into various Met+Cys supply categories revealed that this conclusion applies for marginal, adequate and luxury Met+Cys levels, thus refuting claims that there would be different efficiencies at different dietary Met+Cys levels.

Conclusion

Two studies conducted under Northern European or Middle Eastern conditions confirmed that 100 units of liquid MHA-FA could be replaced by 65 units of DL-Met without compromising performance, regardless of, for example, the choice of ingredients. A more comprehensive evaluation involving 76 pairs of treatments provides evidence that not only can MHA be replaced by DL-Met in a 100:65 ratio with no risk, but also that this conclusion is valid for any general dietary Met+Cys supply status.

Study name	Hedges'g (95% CI)
Elwert et al. (2008)	0.82 (-0.72 to 2.37)
Elwert et al. (2008)	0.00 (-1.48 to 1.48)
Elwert et al. (2008)	0.14 (-1.35 to 1.63)
Elwert et al. (2008)	-0.46 (-1.97 to 1.04)
Hoehler et al. (2005)	0.99 (-0.58 to 2.56)
Hoehler et al. (2005)	1.04 (-0.54 to 2.62)
Hoehler et al. (2005)	-0.70 (-2.23 to 0.83)
Hoehler et al. (2005)	0.12 (-1.36 to 1.61)
Hoehler et al. (2005)	0.16 (-1.59 to 1.92)
Hoehler et al. (2005)	-1.27 (-3.19 to 0.66)
Hoehler et al. (2005)	0.15 (-1.34 to 1.64)
Hoehler et al. (2005)	0.10 (-1.39 to 1.58)
Hoehler et al. (2005)	-0.44 (-1.94 to 1.06)
Hoehler et al. (2005)	0.00 (-1.48 to 1.48)
Hoehler et al. (2005)	0.62 (-0.90 to 2.14)
Lemme et al. (2020)	1.03 (-0.55 to 2.61)
Lemme et al. (2002)	0.11 (-1.65 to 1.87)
Lemme et al. (2002)	1.37 (-0.58 to 3.32)
Lemme et al. (2002)	0.45 (-1.33 to 2.22)
Mandal et al. (2004)	-0.47 (-1.50 to 0.55)
Payne et al (2006)	-0.75 (-1.98 to 0.47)
Sangali et al. (2014)	0.00 (-1.76 to 1.76)
Sangali et al. (2014)	-0.16 (-1.92 to 1.60)
Li et al. (2019)	-0.25 (-1.34 to 0.84)
Li et al. (2019)	-0.37 (-1.39 to 0.65)
Viana et al. (2009)	-0.11 (-1.30 to 1.07)
Goes et al. (2017)	-0.29 (-1.39 to 0.80)
Goes et al. (2017)	0.00 (-1.09 to 1.09)
Murakami et al (2017)	-0.19 (-1.50 to 1.12)
Murakami et al (2017)	0.02 (-1.29 to 1.32)
Murakami et al (2017)	-0.31 (-1.63 to 1.00)
Murakami et al (2017)	-0.16 (-1.47 to 1.15)
Fact&Figures n°15119	0.63 (-0.40 to 1.67)
Overall (P = 0.629)	**–0.01 (-0.17 to 0.20)**
Heterogeneity (I^2=0.00%)	

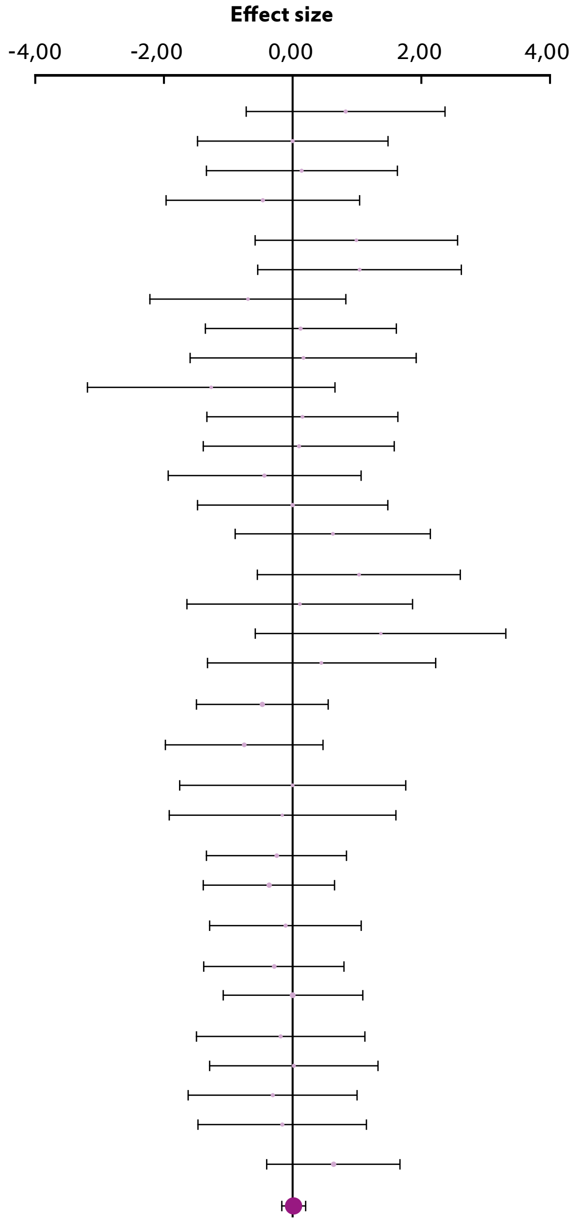

Figure 3. Forest plot showing the effect of dietary replacement of 100 parts MHA with 65 parts of DL-Met on feed conversion ratio of broilers when dietary Met+Cys is below the requirement.

References

1. Lemme, A., V. Naranjo, and J. C. de Paula Dorigam. 2020. Utilization of Methionine Sources for Growth and Met+Cys Deposition in Broilers. Animals : an open access journal from MDPI 10. doi:10.3390/ani10122240.

2. Rychen, G., G. Aquilina, G. Azimonti, V. Bampidis, M. de Lourdes Bastos, G. Bories, A. Chesson, P. S. Cocconcelli, G. Flachowsky, J. Gropp, B. Kolar, M. Kouba, M. López-Alonso, S. López Puente, A. Mantovani, B. Mayo, F. Ramos, M. Saarela, R. E. Villa, P. Wester, L. Costa, N. Dierick, L. Leng, J. Tarrés-Call, and R. J. Wallace. 2018. Safety and efficacy of hydroxy analogue of methionine and its calcium salt (ADRY+®) for all animal species. EFSA journal. European Food Safety Authority 16:e05198. doi:10.2903/j.efsa.2018.5198.

3. Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. Journal of animal science 75:2672–2683. doi:10.2527/1997.75102672x.

4. Sauer, N., K. Emrich, H.-P. Piepho, A. Lemme, M. S. Redshaw, and R. Mosenthin. 2008. Meta-analysis of the relative efficiency of methionine-hydroxy-analogue-free-acid compared with DL-methionine in broilers using nonlinear mixed models. Poultry science 87:2023–2031. doi:10.3382/ps.2007-00514.

5. Hoehler, D., S. Mack, A. Jansman, and J. de Jong. Regression analysis to assess the bioefficacy of different methionine sources in broiler chickens in Proceedings of 26th Poultry Science, Peebles, UK, June 1999.

6. Hoehler, D., A. Lemme, S. K. Jensen, and S. L. Vieira. 2005. Relative Effectiveness of Methionine Sources in Diets for Broiler Chickens. Journal of Applied Poultry Research 14:679–693. doi:10.1093/japr/14.4.679.

7. Elwert, C., E. d. A. Fernandes, and A. Lemme. 2008. Biological Effectiveness of Methionine Hydroxy-analogue Calcium Salt in Relation to DL-Methionine in Broiler Chickens. Asian Australas. J. Anim. Sci 21:1506–1515. doi:10.5713/ajas.2008.80201.

8. Lemme, A., D. Hoehler, J. J. Brennan, and P. F. Mannion. 2002. Relative effectiveness of methionine hydroxy analog compared to DL-methionine in broiler chickens. Poultry science 81:838–845. doi:10.1093/ps/81.6.838.

9. Lemme, A. 2022. DL-Methionine proves more beneficial for broilers. Poultry World Volume 38:38–40.

10. Li, Z., J. C. P. Dorigam, A. Afsar, A. Lemme, G. Da Silva Viana, and E. Musharbash. 2023. Evaluation of methionine sources on performance and carcass traits of broilers at different dietary sulfur amino acid levels under northern European and middle Eastern conditions. Poultry Science Meeting - Book of abstracts:in Press.

11. Herath, H. M. U. L., B. C. Jayawardana, P. D. S. M. Fernando, and P. Weththasinghe. 2023. A meta-analysis of the effects of dietary Spirulina on growth performance of broiler chicken. World's Poultry Science Journal: 1–15. doi:10.1080/00439339.2023.2210325.

12. Agostini, P. S., P. Dalibard, Y. Mercier, P. van der Aar, and J. D. van der Klis. 2016. Comparison of methionine sources around requirement levels using a methionine efficacy method in 0 to 28 day old broilers. Poultry science 95:560–569. doi:10.3382/ps/pev340.

13. Batonon-Alavo, D. I., C. Manceaux, J. T. Wittes, F. Rouffineau, and Y. Mercier. 2023. New statistical approach shows that hydroxy-methionine is non-inferior to DL-Methionine in 35-day old broiler chickens. Poultry science:102519. doi:10.1016/j.psj.2023.102519.

14. Suurmond, R., H. van Rhee, and T. Hak. 2017. Introduction, comparison, and validation of Meta-Essentials: A free and simple tool for meta-analysis. Research synthesis methods 8:537–553. doi:10.1002/jrsm.1260.

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