



Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: <http://www.keaipublishing.com/en/journals/aninu/>
KeAi
 CHINESE ROOTS
 GLOBAL IMPACT

Letter to the Editor

Reply to: “Absorption of methionine sources in animals—is there more to know?” — Yes, there is more to know!



Recently, above mentioned paper compiling and discussing literature concerning the absorption of DL-methionine (DL-Met) and two forms of the hydroxy analogue of methionine (2-hydroxy-4-methylthiobutyric acid [HMTBa], Ca-salt of HMTBa [HMTBa-Ca]) was published in *Animal Nutrition* (<https://doi.org/10.1016/j.aninu.2022.09.004>). We would like to comment on this article regarding the aim of the study as expressed in the introduction: “This critical review provides an understanding of diverging studies and provides insights into how further evaluation should be conducted when the bioefficacy of the two molecules is discussed”.

While methods in physiology research have indeed been further developed and improved in many respects over time, those methods are seldomly appropriate for quantifying impact on performance indicators such as body growth, meat deposition or feed and nutrient utilization. Insofar, research on absorption of methionine or its analogues helps to understand and explain effects determined empirically but would not be suitable to change the conclusions out of growth studies. The mentioned “conflicting” results of bioefficacy studies are indeed a matter of experimental setups which are, in fact, not discussed in the present publication. Moreover, recently the lower bioefficacy of HMTBa and HMTBa-Ca as well as the methodology for determination have been reported and validated, respectively (Elwert et al., 2008; Lemme et al., 2020). After a meticulous examination of literature, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) also concluded that HMTBa shows a lower bioefficacy (75% on molar level) than DLM-Met in monogastric animals which was associated especially with lower efficacy of dimers, trimers and oligomers of HMTBa (Rychen et al., 2018).

In Chapter 2, references and results are reported which should indicate that HMTBa is absorbed to a high degree before feed/digesta enters the duodenum in chicken and other farm animals suggesting high if not identical absorption of this compound compared to DL-Methionine. While it is notable that only references from sponsored research of the co-authors of the present review were considered, authors did not include other studies that reported considerable and higher concentrations of HMTBa

compared to methionine at proximal duodenum in broilers and swine (Drew et al., 2003; Maenz and Engele-Schaan, 1996; Malik et al., 2009) which ingested experimental feeds with labelled methionine sources. Accordingly, the papers concluded a remarkably lower absorption of HMTBa at terminal ileum. In a later chapter (3.1) authors discredit the use of ^3H -labelling and it was suggested that ^3H -labelled compounds would bear the risk of instability. Furthermore, authors devoted more than 60 lines to this subject. In addition, they argued that ^{14}C labelled compounds were also unstable. However, neither research by Saunderson (1985) nor that by Lingens and Molnar (1996) were mentioned. Instructively, these researchers used ^{14}C -labelled methionine and HMTBa or HMTBa-Ca, respectively, and were able to detect and quantify the label in various tissues and the excreta of broiler chickens. Saunderson (1985) reported twice the residual radiation for HMTBa in excreta compared to DL-Met fed broilers which is almost identical to Esteve-Garcia and Austic (1993) findings. Indeed, in Esteve-Garcia and Austic (1993) data would suggest a relative bioefficacy of 64% for HMTBa-Ca compared to DL-Met on weight basis (72% on equimolar comparison). Lingens and Molnar (1996) reported even three-to four-fold residual radiation in excreta of broilers. All these studies would, thus, consistently report substantially higher concentration of HMTBa and HMTBa-Ca compared to the methionine label in excreta (Drew et al., 2003; Lingens and Molnar, 1996; Maenz and Engele-Schaan, 1996; Malik et al., 2009; Saunderson, 1985). Contrary to the data reported by Becquet et al., (2022), these findings are not in agreement with almost complete absorption of HMTBa. Indeed, the detected labels were not necessarily associated with the methionine or HMTBa (Maenz and Engele-Schaan, 1996) and other studies suggested that a large portion of particularly HMTBa has been catabolized by microbiota (Drew et al., 2003; Malik et al., 2009). Therefore, HMTBa available for uptake and further transformation into L-methionine had been shown to be lower than uptake of DL-Met. The entire chapter 3.2 discusses the inappropriateness of the rooster assay for digestibility evaluation as the respective paper by Rostagno and Barbosa (1995) reported a 7% lower apparent digestibility for HMTBa compared to DL-Met and the respective growth study suggested an average bioefficacy of about 66% on weight basis (75% on molar level). In Rostagno and Barbosa (1995), compounds as opposed to labels were analysed and this effect on digestibility would be in addition to the disappearance due to microbial degradation. Whilst established methods are challenged by Becquet et al. (2022), results are consistent despite they were generated from different methodologies. Moreover, the consistent results generated across these studies suggest the methodologies are adequate.

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



<https://doi.org/10.1016/j.aninu.2023.01.010>

2405-6545/© 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

In Chapters 3.3 (Selection of intestinal models), 4 (Oligomer hydrolysis) and 5 (Physiological factors) of the review, authors discuss various in-vitro and ex-vivo models and respective conditions under which research should ideally be performed to investigate absorption of methionine, HMTBa and especially the HMTBa oligomers. In this context, an in-vivo approach reported by Mitchell and Lemme (2008) was overlooked. This research examined the luminal fluid of broilers in order to develop an experimental perfusate used for the intestinal loop technique in broilers that simulated realistic gut conditions. Thus, it could be ensured that concentrations of DL-Met and HMTBa in the intestine were representative for an absorption study. The in-vivo study revealed that absorption of HMTBa was 71% to 83% as efficient as DL-Met absorption. Moreover, the study reported that the dimers of HMTBa, in particular, were almost not absorbed compared to the monomers. Indeed, in this research interaction with gut microbiota were excluded by prior flushing the intestinal segment while all other digestive activities were not restricted.

In Chapter 5 authors emphasize a higher efficiency of HMTBa in context of stressful conditions for broilers especially oxidative stress. However, this conclusion is in contrast with Liu et al. (2019), Maenz and Engele-Schaan (1996), and Wang et al. (2019), which were not mentioned in the above-mentioned review. Rather in contrast, heat exposure did not affect absorption pattern per se during intestinal passage (Maenz and Engele-Schaan, 1996) while research by Liu et al. (2019) and Wang et al. (2019) suggested a higher anti-oxidative efficiency for DL-Met. In addition, research by Zeitz et al. (2018) and Zhang et al. (2018) would suggest improvement of antioxidant status by supplementation of either methionine source compared to Met + Cys deficiency but not necessarily differences between products.

Overall, it appears that the compilation of literature, respective methodologies and chain of argumentation aimed at discrediting studies reporting lower absorption of HMTBa. The impression of a biased review is strengthened due to the omission of a number of easily accessible publications. In conclusion, absorption studies would not allow for revising relative efficiency of methionine sources determined by growth studies and respective validation and challenge trials (Lemme et al., 2020).

Author contributions

Andreas Lemme: conceptualization, Writing-Original Draft
Juliano Cesar de Paula Dorigam: Writing - Review & Editing
Stefan Mack: Writing - Review & Editing

Declaration of conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they belong to Evonik Operations GmbH, Hanau, Germany. The Company produces and sells DL-Methionine.

References

- Drew MD, van Kessel AG, Maenz DD. Absorption of methionine and 2-hydroxy-4-methylthiobutanoic acid in conventional and germ-free chickens. *Poultry Sci* 2003;82(7):1149–53.
- Elwert C, de Abrou Fernandes E, Lemme A. Biological effectiveness of methionine hydroxy-analogue calcium salt in relation to DL-Methionine in broiler chickens. *Asian-Australas J Anim Sci* 2008;21(10):1506–15.
- Esteve-Garcia E, Austic RE. Intestinal absorption and renal excretion of dietary methionine sources by growing chicken. *J Nutr Biochem* 1993;576–87.
- Lemme A, Naranjo V, de Paula Dorigam JC. Utilization of methionine sources for growth and Met+Cys deposition in broilers. *Animals* 2020;10(12).
- Lingens G, Molnar S. Studies on metabolism of broilers by using ¹⁴C-labelled DL-methionine and DL-methionine hydroxy analogue calcium salt. *Arch Anim Nutr* 1996;49:113–24.
- Liu G, Magnuson AD, Sun T, Tolba SA, Starkey C, Whelan R, et al. Supplemental methionine exerted chemical form-dependent effects on antioxidant status, inflammation-related gene expression, and fatty acid profiles of broiler chicks raised at high ambient temperature. *J Anim Sci* 2019;97(12):4883–94.
- Maenz DD, Engele-Schaan CM. Methionine and 2-hydroxy-4-methylthiobutanoic acid are partially converted to nonabsorbed compounds during passage through the small intestine and heat exposure does not affect small intestinal absorption of methionine sources in broiler chicks. *J Nutr* 1996;126(5):1438–44.
- Malik C, Hoehler D, Rademacher M, Drew MD, van Kessel AG. Apparent absorption of methionine and 2-hydroxy-4-methylthiobutanoic acid from gastrointestinal tract of conventional and gnotobiotic pigs. *Animal* 2009;3(10):1378–86. <https://doi.org/10.1017/S1751731109990267>.
- Mitchell MA, Lemme A. Examination of the composition of the luminal fluid in the small intestine of broilers and absorption of amino acids under various ambient temperatures measured *in vivo*. *Int J Poultry Sci* 2008;7(3):223–33.
- Rostagno HS, Barbosa WA. Biological efficacy and absorption of dl-methionine hydroxy analogue free acid compared to dl-methionine in chickens as affected by heat stress. *Br Poultry Sci* 1995;36:303–12.
- Rychen G, Aquilina G, Azimonti G, Bampidis V, Lourdes Bastos M de, Bories G, et al. Safety and efficacy of hydroxy analogue of methionine and its calcium salt (ADRY+®) for all animal species. *EFSA journal*. European Food Safety Authority 2018;16(3):e05198. <https://doi.org/10.2903/j.efsa.2018.5198>.
- Saunderson CL. Comparative metabolism of L-methionine, DL-methionine and DL-2-hydroxy 4-methylthiobutanoic acid by broiler chicks. *Br J Nutr* 1985;54(3):621–33. <https://doi.org/10.1079/bjn19850149>.
- Wang Y, Yin X, Yin D, Lei Z, Mahmood T, Yuan J. Antioxidant response and bioavailability of methionine hydroxy analog relative to DL-methionine in broiler chickens. *Animal nutrition* 2019;5(3):241–7. <https://doi.org/10.1016/j.aninu.2019.06.007>.
- Zeitz JO, Mohrmann S, Fehse L, Most E, Helmbrecht A, Saremi B, et al. Tissue and plasma antioxidant status in response to dietary methionine concentration and source in broilers. *J Anim Physiol Anim Nutr* 2018;102(4):999–1011. <https://doi.org/10.1111/jpn.12909>.
- Zhang S, Gilbert ER, Saremi B, Wong EA. Supplemental methionine sources have a neutral impact on oxidative status in broiler chickens. *J Anim Physiol Anim Nutr* 2018;102(5):1274–83. <https://doi.org/10.1111/jpn.12946>.

Andreas Lemme*, Juliano Cesar de Paula Dorigam, Stefan Mack
 Evonik Operations GmbH, Hanau, Germany

* Corresponding author.

E-mail address: andreas.lemme@evonik.com (A. Lemme).

6 December 2022
 Available online 9 February 2023

Absorption of methionine sources in animals – is there more to know? – Clarification

Our publication on absorption of methionine sources (Becquet et al., 2023a) is a thorough analysis of 129 references dealing with absorption of sources of methionine, with the objective to critically evaluate the methodologies used and their impact on the conclusions of the studies. As the focus of our publication was on the evaluation of absorption, efficacy studies and reports not having information on absorption were not considered (Elwert et al., 2008; Rychen et al., 2008). Furthermore, the search was limited to 2019 as indicated in the introduction of the publication, hence studies or reports published thereafter were not considered, such as Lemme et al. (2020).

The publication (Becquet et al., 2023a) should enable readers to consider the different aspects of the methodology, when setting studies to evaluate or when reading studies evaluating the absorption of the methionine sources.

At no place does our review (Becquet et al., 2023a) mention any bioequivalence values between HMTBa, HMTBa-Ca and DL-methionine as it focuses on absorption. It is therefore interesting to note that the first part of the Letter to the Editor (Lemme et al., 2023) refers specifically to this aspect. It is well known that bioequivalence between HMTBa, HMTBa-Ca and DL-methionine remains a controversial subject, despite years of research. It was not in the scope of our review and hence efficacy studies were not covered. But the Letter to the Editor (Lemme et al., 2023) only referenced selected efficacy studies, favoring DL-Methionine (Elwert et al., 2008; Lemme et al., 2020) and the EFSA opinion (Rychen et al., 2008), but omitting to include studies supporting equal bioefficacy such as Batonon-Alavo et al. (2023) and Uddin et al. (2022), which is a meta-analysis.

Furthermore, the authors of the Letter to the Editor (Lemme et al., 2023) indicate that our review does not include studies such as Drew et al. (2003), Maenz and Engele-Schaan (1993) and Malik et al. (2009) without paying due attention to the list of references at the end of our review. Yes, the publications from Lingens and Molna (1996) and Saunderson (1985) were indeed not discussed in-depth in this review as they addressed metabolism of methionine sources rather than absorption but were included in the list of references in our publication on the metabolism of sources of methionine (Becquet et al., 2023b), the Part II of our review. The authors of the Letter to the Editor (Lemme et al., 2023) cited that both references reported higher residual radiation for HMTBa in excreta, suggesting lower bioefficacy compared with DL-methionine without reporting higher residual radiation for DL-methionine in excreta in comparison to HMTBa (Gordon and Sizer, 1965; Saroka and Combs Jr, 1968), whereas all these studies were unbiasedly included and discussed in our review of the metabolism assessments of methionine sources (Becquet et al., 2023b). Finally, the study from Mitchell and Lemme (2008) was not considered in our review because the proposed method (only presented in this paper), did not have sufficient information to allow a meaningful comparison with other studies.

The highlights of the Letter to the Editor “The authors are concerned regarding the quality and scientific standards of a recent publication by Becquet et al.” is not supported by the content of the Letter to the Editor. As described above, some elements of the Letter to the Editor are debatable. Hence, the authors of Becquet et al. (2023a) also question the quality and scientific approach and standards of the Letter to the Editor (Lemme et al., 2023), which did not go through a rigorous review process, comparable to our two publications (Becquet et al., 2023a, 2023b). While the authors of the letter to the Editor, which provides confusion rather than value to the scientific community, our publication deep dives in

the comparison of the methodologies used and their relevance due to the different chemical nature of HMTBa and HMTBa-Ca compared to DL-methionine. The latter approach provides key foundations to the readers for properly evaluating the published studies and preparing future studies.

References

- Batonon-Alavo DI, Manceaux C, Wittes JT, Rouffineau F, Mercier Y. New statistical approach shows that hydroxy-methionine is noninferior to DL-methionine in 35-day-old broiler chickens. *Poultry Sci* 2023;102(102519):1–9.
- Becquet P, Vazquez-Anon M, Mercier Y, Batonon-Alavo DI, Yan F, Wedekind K, Mahmood T. Absorption of methionine sources in animals - is there more to know? *Animal Nutrition* 2023a;12:159–70. <https://doi.org/10.1016/j.aninu.2022.09.004>.
- Becquet P, Vazquez-Anon M, Mercier Y, Wedekind K, Mahmood T, Batonon-Alavo DI, Yan F. A systematic review of metabolism of methionine sources in animals: one parameter does not convey a comprehensive story. *Animal Nutrition* 2023b;13:31–49. <https://doi.org/10.1016/j.aninu.2023.01.009>.
- Drew MD, van Kessel AG, Maenz DD. Absorption of methionine and 2-hydroxy-4-methylthiobutanoic acid in conventional and germ-free chickens. *Poultry Sci* 2003;82(7):1149–53.
- Elwert C, de Abreu Fernandez E, Lemme A. Biological effectiveness of methionine hydroxy-analogue calcium salt in relation to DL-methionine in broiler chickens. *Asian Australian Journal of Animal Science* 2008;21(10):1506–15.
- Gordon R, Sizer IW. Conversion of methionine hydroxy analogue to methionine in the chick. *Poultry Sci* 1965;44(3):673e8.
- Lemme A, de Paula Dorigam JC, Mack S. Reply to 'Absorption of methionine sources in animals - is there more to know?' - yes there is more to know. *Animal Nutrition* 2023.
- Lemme A, Naranjo V, de Paula Dorigam JC. Utilization of methionine sources for growth and Met+Cys deposition in broilers. *Animals* 2020;10(12). <https://doi.org/10.3390/ani10122240>.
- Lingens G, Molnar S. Studies on the metabolism of broilers by using ¹⁴C-labelled DL-methionine and D-methionine hydroxyanalogue calcium salt. *Arch Anim Nutr* 1996;49(2):113–24.
- Maenz DD, Engele-Schaan CM. Methionine and 2-hydroxy-4-methylthiobutanoic acid are partially converted to nonabsorbed compounds during passage through the small intestine and heat exposure does not affect small intestinal absorption of methionine sources in broiler chicks. *J Nutr* 1993;126(5):1438–44.
- Malik G, Hoehler D, Rademacher M, Drew MD, van Kessel AG. Pparent absorption of methionine and 2-hydroxy-4-methylthiobutanoic acid from gastrointestinal tract of conventional and gnotobiotic pigs. *Animal* 2009;3(10):1178–786.
- Mitchell MA, Lemme A. Examination of the composition of the luminal fluid in the small intestine of broilers and absorption of amino acids under various ambient temperatures measured in vivo. *Int J Poultry Sci* 2008;7(3):223–33.
- Rychen G, Aquilina G, Azimonti G, Bampidis V, Lourdes Bastos M, Borjes G, et al. Safety and efficacy of hydroxy analogue of methionine and its calcium salt (ADRY+(R)) for all animal species. *EFSA J* 2008;16(3):e05198. <https://doi.org/10.2903/j.efsa.2018.5198>.
- Saroka JM, Combs Jr GF. Studies on the renal excretion of the hydroxyl analogue of methionine by the chick. *Poultry Sci* 1968;65(7):1375e82.
- Saunderson CL. Comparative metabolism of L-methionine, DL-methionine and DL-2-hydroxy 4-methylthiobutanoic acid by broiler chicks. *Br J Nutr* 1985;54(3):621–33.
- Uddin ME, van Lingen HJ, da Silva Pires PG, Batonon-Alavo DI, Rouffineau F, Kebreab E. Evaluating growth response of broiler chickens fed diets supplemented with synthetic DL-methionine or DL-hydroxymethionine: a meta-analysis. *Poultry Sci* 2022;101:101762.

Philippe Becquet*
IMAA, Brussels, Belgium

Mercedes Vazquez-Anon
Novus International, Saint Charles, Missouri, USA

Yves Mercier, Dolores I. Batonon-Alavo
Adisseo France SAS, Antony, France

Frances Yan, Karen Wedekind
Novus International, Saint Charles, Missouri, USA

Tahir Mahmood
Adisseo France SAS, Antony, France

* Corresponding author.