Recent broiler trial confirms 2018 EFSA (European Food Safety Authority) scientific opinion on methionine sources while validating the experimental approach

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TAKE HOME MESSAGE

- The present study was conducted to determine the relative biological availability (RBA) of different methionine sources. The results revealed average RBA of 58 – 65% for liquid methionine hydroxy analogue (MHA-FA) and 54 - 65% for 65% purity diluted MetAMINO® (DLM65), respectively, compared to MetAMINO® for various performance parameters.
- The results demonstrated that the RBA of liquid MHA-FA is significantly lower than its active content of 88 % and is therefore consistent with the 2018 EFSA (European Food Safety Authority) scientific opinion of 66% on a product basis (75 % on equimolar basis).
- The average RBA of 61% for DLM65 was close to the expected value of 65% (because of dilution to 65 % purity) and thus confirms the appropriateness of using the simultaneous dose-response approach to determine the RBA of nutrient sources.
- The results of this study complement the findings of several other studies with respect to the RBA of both liquid MHA-FA and DLM65.

AN ONGOING DEBATE ON THE RELATIVE BIOLOGICAL AVAILABILITY (RBA) OF METHIONINE SOURCES LED TO A NEW TRIAL WITH BROILERS.

Commercial poultry diets are routinely supplemented with methionine sources to precisely meet their Met+Cys specifications. Around two-thirds of the global market is supplied with DL-methionine (DLM) and one third with DL-methionine hydroxy analogue products, which are mainly in the liquid free acid form (MHA-FA). Despite the fact that DLM is the most preferred Met source globally, the question about the nutritive value of liquid MHA-FA being characterized by the RBA is still a critical factor for both cost-effective purchasing and optimization of feed costs.

Various literature reviews have reported a RBA of about 65 % for liquid MHA-FA compared to DLM in broilers on product-to-product basis (Jansman et al., 2003; Lemme et al., 2012). More recently, the European Food Safety Authority (EFSA, 2018), which examines and assesses dossiers for product registration in the European Union, released a scientific opinion on liquid MHA-FA and its calcium salt. This opinion stated a RBA of 75% on equimolar basis which is equivalent to 66% on product-to-product basis for liquid MHA-FA. While this 65% RBA of MHA products compared to DLM has been evaluated and confirmed in many field and university trials (Lemme et al., 2012), the experimental setup to determine the RBA has been a matter of recent discussion (Vazquez-Anon et al, 2006). In order to provide further evidence that the methodology of a simultaneous dose-response feeding trial is a valid approach and the methodology of choice, a test product can be included from which the RBA is known *a priori.* Diluting DLM (e.g., with starch) to a purity of 65% (65 parts DLM and 35 parts starch to provide DLM65) would be such a product in which the RBA would be assumed to be close to 65 % because of its purity. Although several poultry experiments with this approach have already been published (Lemme et al., 2002; Hoehler et al., 2005, Elwert et al., 2008), data with modern broiler genetics are needed. Therefore, a study was conducted at Schothorst Feed Research in the Netherlands to determine the RBA of liquid MHA-FA relative to MetAMINO® (DLM) for common performance parameters and to validate the suitability of the multi-exponential regression analysis for estimating the RBA by including DLM65 as an internal standard.

A SIMULTANEOUS DOSE-RESPONSE FEEDING TRIAL WITH 5 GRADED LEVELS OF 3 METHIONINE SOURCES WAS CONDUCTED.

A total of 1,920 d-old male Ross 308 broilers were allocated to 96 floor pens of 20 chicks each. Each pen ($2m^2$) had one feeder, a line of drinking nipples, and wood shavings as bedding material. Ambient temperature and light regime were in line with commercial standards and complying with legislation. Feeds were formulated to meet or exceed the ideal AA profile of recommendations (AMINOChick® 2.0), except for digestible Met and Met+Cys (Table 1). Main ingredients were analyzed by near infrared spectroscopy (AMINONIR®), and diet formulations were adjusted accordingly. Broilers were fed starter (d 0 to 11), grower (d 11 to 28), and finisher (d 28 to 35) diets. Each phase was comprised of 16 treatments, including a basal diet deficient in dig. Met+Cys without supplemental Met and 5 increasing levels of either MetAMINO®, liquid MHA-FA, or DLM65. Met sources were added in all phases on weight/weight basis at 0.40, 0.80, 1.20, 2.10 and 3.00 g/kg. Analyzed values of the experimental diets from all phases were in close agreement with the calculated values (Table 1). Recovery rates ranged from 97 to 109 % for total Met+Cys and Lys. Starter feeds were produced in crumbles, while grower and finisher diets were pelleted. Feed and water were supplied *ad libitum* throughout the experimental period.

Table 1: Dietary ingredients and calculated nutrient composition (g/kg) of the experimental basal diets

	Starter d 0 to 11	Grower d 11 to 28	Finisher d 28 to 35
Ingredients			
Wheat	286.1	364.6	366.0
Corn	250.0	208.8	200.0
Soybean meal	323.7	255.8	218.6
Peas	50.0	100.0	139.4
Corn gluten meal	18.2	---	---
Limestone	14.5	11.6	9.0
Soybean oil	14.4	11.3	17.3
Pork fat lard	19.4	28.6	34.1
Monocalcium phosphate	7.6	4.3	1.3
Salt	3.3	2.5	2.6
Lysine-HCl (78 %)	2.7	1.8	1.4
ThreAMINO® (98.5 %)	1.1	0.9	0.7
ValAMINO® (96.5 %)	0.7	0.5	0.3
Phytase	3.3	3.3	3.3
Sodium bicarbonate	---	1.0	1.0
Premix (coccidiostat + NSP enzyme)	5.0	5.0	5.0
Calculated content[1]			
AMEn (kcal/kg)	2,900	2,975	3,075
AMEn (MJ/kg)	12.13	12.45	12.87
Crude protein	233.5 (237.3)	205.2 (210.7)	194.2 (198.7)
Crude fibre	23.4	24.4	25.1
Dig. Lys[1]	12.6	10.7	9.9
Dig. Met	3.04	2.56	2.40
Dig. **Met+Cys**	**6.17**	**5.35**	**5.05**
Dig. Thr	8.19	6.96	6.44
Dig. Val	10.00	8.56	7.92
Dig. Ile	8.55	7.38	6.93
Dig. Arg	13.34	11.93	11.34
Dig. Trp	2.53	2.24	2.08
Total Lys[2]	14.1 (14.6)	12.0 (12.4)	11.2 (11.9)
Total Met+Cys	7.1 (7.2)	6.2 (6.2)	5.8 (6.3)
Total Thr	9.5 (9.7)	8.1 (8.6)	7.6 (7.8)
Total Val	11.4 (11.5)	9.8 (9.8)	9.1 (9.6)
Calcium	0.79	0.61	0.46
Retainable phosphorous	0.38	0.31	0.25
DEB (mEq/kg)	226	221	211

[1]Formulation based on apparent fecal digestibility (AFD) according to research institute. [2]Values in parenthesis represent analyzed values.

BROILERS RESPONDED SIGNIFICANTLY TO METHIONINE SOURCES.

Responses of broilers to increasing levels of either MetAMINO®, liquid MHA-FA, or DLM65 are shown in Table 2 as well as in Figures 1 & 2. Birds fed the unsupplemented basal diets grew only 67% as efficiently as suggested by Ross 308 Broiler Objectives (2014), while body weight gains (BWG) achieved with highest supplementation levels were about 12% higher than broiler objectives with methionine products. Relative to the basal treatment, the highest addition (3.00 g/kg) of MetAMINO®, liquid MHA-FA, or DLM65 significantly improved BWG by 70, 67, and 70% and reduced feed conversion ratio (FCR) by 22, 22, and 23%, respectively. Similarly, carcass yield (CY) was improved by 13, 11 and 13% and breast muscle yield (BMY) by 62, 60 and 63% with MetAMINO®, liquid MHA-FA, or DLM65, respectively. This suggests that, if enough product is added to feed, maximum performance can also be achieved with a product with lower methionine content, such as DLM65. Therefore, comparing methionine sources above the level which is required would not lead to a reliable conclusion on their biological availability. Incremental supplementation of Met sources improved performance in a non-linear manner following the law of diminishing returns (Figures 1 & 2). In addition, data points of three or more supplementation levels of each test product are located in the curve-linear range of the response curve, while all products indicated a common plateau. Therefore, all important prerequisites for multi-exponential regression to determine RBA, as described by Jansman et al. (2003) and Sauer et al. (2014), were met. Therefore, per definition, studies in which the first inclusion level of products almost describe the asymptote of the response are not appropriate for distinction between RBA of methionine sources.

Table 2: Performance data of broilers fed different Met sources from 0 to 35 d of age

Met source	Added product	Body weight gain		Feed intake		Feed conversion ratio		Carcass yield		Breast meat yield	
		g	Rel.*	g	Rel.*	g/g	Rel.*	% of BW	Rel.*	% of BW	Rel.*
Basal	0.00	1492^{a}	100	2809^{a}	100	1.885^{h}	100	58.1^{a}	100	12.3^{a}	100
MetAMINO®	0.40	2212^{d}	148	3550de	126	1.605^{e}	85	62.1^{c}	107	15.4^{cd}	125
MetAMINO®	0.80	2347^{e}	157	3649^{efg}	130	1.555^{cd}	83	63.8^{def}	110	17.4^{efg}	141
MetAMINO®	1.20	2455^{f}	165	3750^{g}	134	1.528^{bc}	81	66.1^{i}	114	18.5^{hi}	150
MetAMINO®	2.10	2551^{f}	171	3716^{fg}	132	1.457^{a}	77	65.4^{ghi}	113	20.4^{k}	165
MetAMINO®	3.00	2542^{f}	**170**	3726^{fg}	**133**	1.467^{a}	**78**	65.8^{hi}	**113**	20.0^{k}	**162**
MHA-FA	0.40	1896^{c}	127	3316^{bc}	118	1.750^{f}	93	61.6^{c}	106	14.7^{bc}	119
MHA-FA	0.80	2179^{d}	146	3438^{cd}	122	1.578^{de}	84	62.9^{cde}	108	16.4^{de}	133
MHA-FA	1.20	2354^{e}	158	3658^{efg}	130	1.554^{cd}	82	64.2^{efgh}	111	17.3^{ef}	140
MHA-FA	2.10	2489^{f}	167	3709^{fg}	132	1.490^{ab}	79	64.1^{efg}	110	18.4^{ghi}	150
MHA-FA	3.00	2496^{f}	**167**	3673^{efg}	**131**	1.472^{a}	**78**	65.0^{fghi}	**111**	19.7^{jk}	**160**
DLM65	0.40	1756^{b}	118	3181^{b}	113	1.813^{g}	96	60.0^{b}	103	14.0^{b}	114
DLM65	0.80	2226^{d}	149	3592^{ef}	128	1.614^{e}	86	62.4^{cd}	107	15.7^{cd}	128
DLM65	1.20	2328^{e}	156	3708^{fg}	132	1.593^{de}	85	64.7^{fghi}	111	17.6^{fgh}	143
DLM65	2.10	2501^{f}	168	3726^{fg}	133	1.491^{ab}	79	65.4^{fghi}	112	18.9^{ij}	154
DLM65	3.00	2529^{f}	**170**	3688^{fg}	**131**	1.459^{a}	**77**	65.5^{ghi}	**113**	20.0^{k}	**163**
LSD		100.4		137.2		0.042		1.65		1.04	
P-value		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

LSD: Least significant difference.
a-k Values without a common superscript within a column differ significantly (P ≤ 0.05).
Rel*: Relative response of each dietary treatment compared to the basal diet, which was set as 100 %.

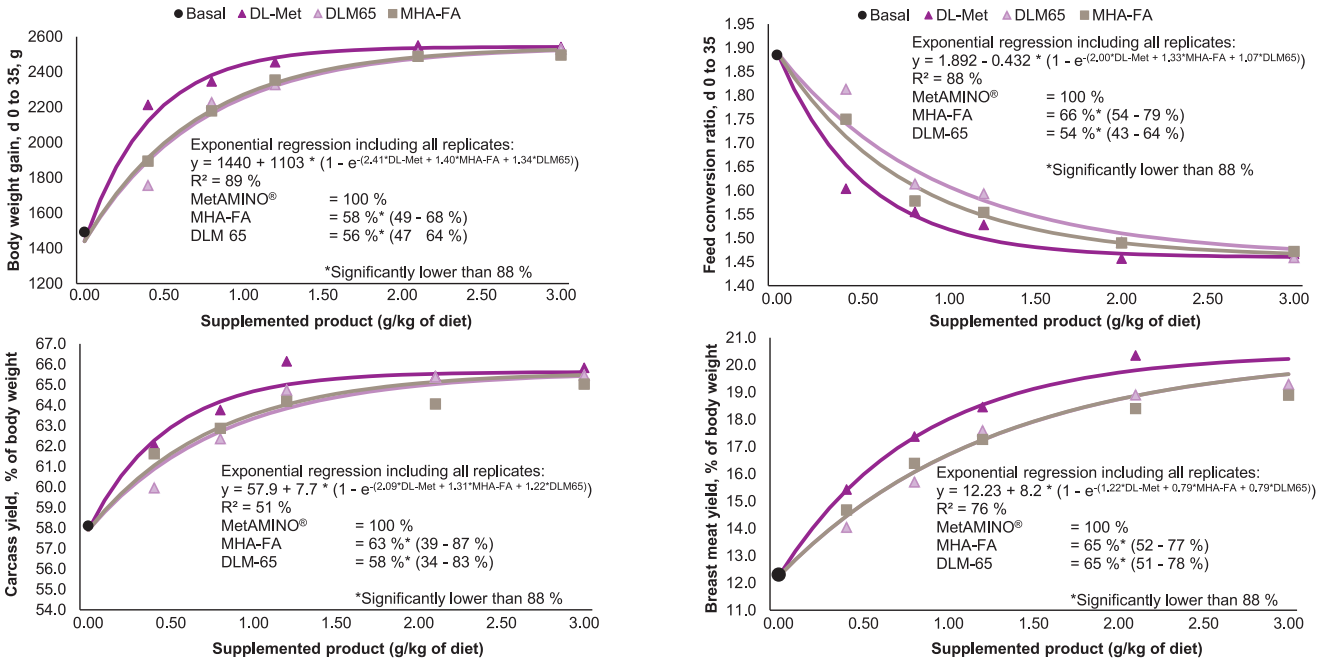

Figure 1: Comparison with MetAMINO® revealed a significantly lower RBA for MHA-FA and DLM65 in regards to body weight gain, feed conversion ratio , carcass yield, and breast meat yield in male broilers from 0 to 35 days of age.

RELATIVE BIOAVAILABILITY OF LIQUID MHA-FA WAS SIGNIFICANTLY LOWER COMPARED TO METAMINO®.

Multi-exponential regression analysis revealed that liquid MHA-FA was only 58, 66, 63 and 65% as efficacious as MetAMINO® for BWG, FCR, CY and BMY, respectively. Similarly, the estimated RBA of DLM65 for these parameters were 56, 54, 58 and 65%, respectively. It is concluded that the estimated RBA figures were both in a narrow range and very similar for liquid MHA-FA and DLM65. Furthermore, the RBA figures for DLM65 were close to 65%, which was the expected value for a product with 65% purity. However, these figures were numerically lower than 65% for BWG, FCR, and CY due to biological variation, especially with the first inclusion level. Overall, the similarity of RBA figures for liquid MHA-FA and DLM65 for single performance parameters indicate that there were not substantial differences in biological effectiveness between these two products. The latter conclusion is confirmed by multi-simultaneous regression analysis of European production efficiency factor (EPEF) data. The EPEF considers the final body weight, feed conversion ratio, and survival of the birds and serves as an economic indicator. The RBA of liquid MHA-FA and DLM65 for EPEF were 62 and 59%, suggesting again that MetAMINO® diluted to a purity of 65% was as efficient as liquid MHA-FA. Moreover, all RBA estimates for both liquid MHA-FA and DLM65 were significantly different from 88%, which is the content of active substance of liquid MHA-FA.

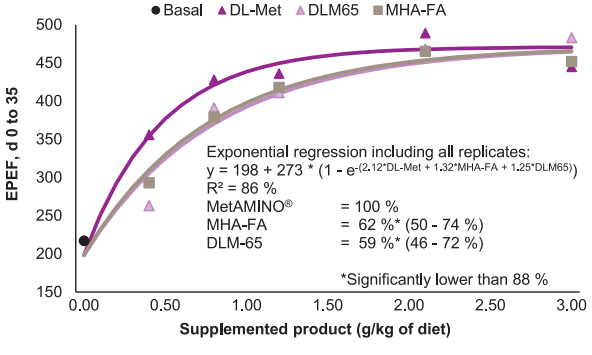

Figure 2: Responses of 1-35 day old male broilers to graded levels of MetAMINO®, liquid MHA-FA, and DLM65 on the European Production Efficiency Factor (EPEF)* revealed significantly lower RBA of liquid MHA-FA and DLM65 compared to MetAMINO®, while liquid MHA-FA and DLM65 did not differ in their RBA.

*** ([Survival (%) x final body weight (kg)] / [age (days) x FCR (kg/kg)]) x 100**

The findings of the current study are in line with the results of previous studies, which included DLM65 treatments as well (Table 3). Accordingly, the average RBA from results across performance parameters, such as body weight gain, feed conversion ratio, and breast meat yield, obtained from six experiments was 63 % for DLM65 and 62% for liquid MHA-FA, confirming an RBA of close to 65% for liquid MHA-FA. It also confirms an RBA of close to 65% for DLM65, which is therefore confirming the validity of the method to determine RBA of Met sources. Each single RBA figure shown in Table 3 was significantly different from 88%, which also confirms recent conclusions of the European Food Safety Authority (EFSA, 2018) that there is convincing evidence that the RBA of MHA products is lower than 88% and is about 66% on product to product basis (75% on equimolar basis).

Table 3: Compilation of feeding experiments, which included liquid MHA-FA and diluted MetAMINO® (DLM65), to determine the biological availability relative to MetAMINO®

Study		Weight gain		Feed conversion ratio		Breast meat yield	
	Year	DLM65	liquid MHA-FA	DLM65	liquid MHA-FA	DLM65	liquid MHA-FA
1 *	1999	59 %	57 %	66 %	58 %		
2 *	2002	60 %	68 %	57 %	67 %	69 %	64 %
3 *	2005	67 %	64 %	59 %	67 %		
4 *	2005	69 %	63 %	79 %	73 %		
5 *	2005	59 %	65 %	47 %	49 %		
Schothorst*	**2018**	**58 %**	**56 %**	**66 %**	**54 %**	**65 %**	**65 %**
Average		62.0 %	62.2 %	62.3 %	61.3 %	67.0 %	64.5 %
Overall weighted average across all criteria				**DL-Met 65 62.9 %**	**liquid MHA-FA 62.1 %**		

* 1: TNO-ILOB, The Netherlands, Degussa Facts&Figures Poultry 6, 1999; 2: QPRDC, Australia, in: Lemme et al., 2002; 3: Research Centre Foulum, Denmark 4: Mexican Integrator 5: University of Arkansas, all in Hoehler et al., 2005

THE OUTCOME OF THESE STUDIES CAN BE CHALLENGED UNDER ANY COMMERCIAL CONDITIONS.

While there is a large body of data evaluating the RBA of methionine sources (including those presented above which provide evidence on the validity of the method), there is still a discussion on implementation into practical poultry nutrition. Evonik offers to conduct tests to challenge the RBA of 65% under any production conditions. Such test can be run with MetAMINO® replacing MHA-products in a ratio 65:100 as well as with PROXYMet™ (MetAMINO® with 65% purity) replacing MHA-products in a 1:1 ratio. In either case, the result will be the same: Same performance obtained from animals fed the different methionine sources, but economic savings observed when MetAMINO® is used.

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