Asian-Aust. J. Anim. Sci.
Vol. 21, No. 10 : 1506 - 1515
October 2008

www.ajas.info

Biological Effectiveness of Methionine Hydroxy-analogue Calcium Salt in Relation to DL-Methionine in Broiler Chickens

C. Elwert*, E. de Abreu Fernandes[1] and A. Lemme[2]
Feedtest, Gottgau 3b, 06193 Löbejün, Germany

ABSTRACT : Two feeding trials were conducted to assess the relative bioavailability (RBV) of methionine hydroxy analogue calcium salt (MHA-Ca) in comparison to DL-methionine (DL-Met). Male Ross 308 (1-38 days) and Cobb 500 chickens (1-42 days) were used in studies 1 and 2, respectively. Experimental diets based on wheat and soybean meal or sorghum and soybean meal were fed during three phases. In both experiments graded levels of DL-Met and MHA-Ca were supplemented to Met+Cys deficient basal diets. Additionally, in experiment 1, increasing levels of a DL-Met preparation diluted with corn starch to 65% purity (DL-Met65) were supplemented. Birds were kept in floor pens and feed and water were available *ad libitum*. Body weights and feed consumption were recorded at the beginning and end of the experimental periods and weight gain and feed efficacy were computed subsequently. At the end of the experiments, a number of birds were slaughtered for carcass evaluation (dressing percentage, breast meat yield). Dose response data were analysed by both ANOVA and nonlinear common plateau asymptotic regression. In both experiments birds responded significantly to increasing levels of either methionine source. However, RBV of MHA-Ca compared to DL-Met was markedly (in many cases significantly) below 84%, the value which would have been expected from MHA-Ca's chemical characteristics. Excluding some extremely low RBV figures of trial 2, RBV of MHA-Ca averaged to about 63% in relation to DL-Met. In addition, supplementation of DL-Met65 allowed confirmation of nonlinear common plateau asymptotic regression to be suitable to determine RBV. (**Key Words :** Broiler, Methionine Sources, Relative Efficiency, Performance)

INTRODUCTION

Supplementing methionine sources to broiler diets in order to balance the dietary protein in accordance to the broilers' demand or the economic optimum is common practice. Over the last 15 years, DL-methionine (DL-Met) and liquid MHA-FA which is the hydroxy analogue of DL-Met were alternative sources. The question about the biological effectiveness (BE) or relative bioavailability (RBV) of liquid MHA-FA was investigated in a large number of experiments. Recently, two extensive literature surveys were performed suggesting a RBV of about 65% for liquid MHA-FA in broilers in relation to DL-Met on "as is" basis being equivalent to 74% on molar comparison (Jansman et al., 2003; Lemme and Petri, 2003). Sauer et al. (2007), applying the approach of meta-analysis, reported a RBV of about 70% on "as is" basis. All these surveys used the same principle for RBV determination which is describing the biological response of broilers to graded supplementation levels of a product in relation to a reference product by exponential functions. Starting points and asymptotes of the curves for both products are the same and thus the only difference between the curves is the steepness. Relating the regression coefficient of the DL-Met equation to that of the liquid MHA-FA equation releases the RBV. Kratzer and Littell (2006) recently questioned this approach and postulated that the asymptotes of the response curves to supplemental MHA-FA and DL-Met are different and, consequently, RBV cannot be determined by the common plateau approach whereas investigations by Piepho (2006) and Sauer et al. (2007) supported the hypothesis of a common plateau for methionine source responses in broilers to be valid.

Recently, the calcium salt of the methionine hydroxy analogue (MHA-Ca) has been re-introduced in the market. For this product there is basically the same question about its relative bioavailability (RBV) compared to DL-Met and appropriate methods for RBV measurement as for liquid

* Corresponding Author: C. Elwert. Tel: +49-177-6567921, E-mail: elwert@feedtest.de
[1] UFU, Av. Pará 1720 Bloco 2T, 314000-902 Uberlândia, Brazil.
[2] Evonik Degussa GmbH, Rodenbacher Chaussee 4, 63457 Hanau-Wolfgang, Germany.
Received April 10, 2008; Accepted June 21, 2008

Table 1. Composition of the basal diets, calculated energy content and analyzed crude protein and amino acid concentrations, Trial 1

Ingredients (g/kg)	Starter (days 0-10)	Grower (days 11-27)	Finisher (days 28-38)
Wheat	202.2	465.6	594.7
Corn	200.0	-	-
Soybean meal (48)	389.7	291.6	227.6
Peas	100.0	124.1	159.6
Soybean oil	59.7	79.4	77.8
Biolys	1.09	1.14	1.34
L-threonine	0.40	0.44	0.62
Calciumcarbonate	14.3	12.5	12.7
Monocalciumphosphate	22.0	16.0	16.4
Salt (NaCl)	2.65	2.70	2.70
Natriumbicarbonate	2.93	1.51	1.52
Vitamin-mineral-enzyme premix	5.00	5.00	5.00
AME_N (kcal/kg)	3,010	3,170	3,200
AME_N (MJ/kg)	(12.6)	(13.3)	(13.4)
Crude protein	248	218	198
Methionine	3.3	3.0	2.7
Met+Cys	6.9	6.5	5.8
Lysine	14.3	13.2	11.3
Threonine	9.4	8.7	7.6
Arginine	17.2	15.1	13.7

MHA-FA. Therefore, new studies were designed to determine RBV for MHA-Ca in broilers as the majority of existing MHA-Ca studies has been performed between the 1960s and early 1990s. A literature survey based on 65 data sets (weight gain, feed conversion) from 33 experiments conducted in this time revealed an average RBV of 63.8% for MHA-Ca on product basis which is similar to 63.2% found for liquid MHA-FA (Lemme, 2004).

The studies reported here follow the exponential approach which has been proven to be a proper mathematical model by using an internal standard from which the RBV was known a priori (Lemme et al., 2002; Hoehler et al., 2005; Payne et al., 2006). Both studies were multiple dose response experiments with growing broilers and were conducted in Germany and Brazil. In trial 1, the nonlinear common asymptote regression approach as described by Littell et al. (1997) was validated by using diluted DL-Met (65%; DL-Met65) serving as internal standard, trial 2 was designed as a typical dose response study with increasing supplementation levels of MHA-Ca compared to DL-Met.

MATERIALS AND METHODS

Trial 1 was conducted at the facilities of feedtest, Germany. A commercial diet based on wheat, soybean meal, and peas was formulated to meet the animals' requirements except for Met+Cys for the starter (days 1 to 10), grower (days 11 to 27), and finisher (days 28-38) phase (Table 1). These basal diets had Met+Cys contents of 6.9, 6.5, and 5.8 g/kg, and were each supplemented with 5 graded levels (0.3, 0.6, 1.0, 1.5 and 2.1 g/kg diet) of either DL-Met, MHA-Ca, or DL-Met diluted with cornstarch to a purity of 65% (DL-Met65). The supplemented levels were confirmed by analysis. If the exponential common plateau regression approach according to Littell et al. (1997) were valid, the RBV for DL-Met65 should be determined at about 65%. Therefore, the DL-Met65 treatments served as an internal standard of the chosen statistical analysis. Diets contained a coccidiostat in the starter and grower but not in finisher period and were prepared at a dedicated compound feed plant (Research Diet Services BV, Hoge Maat 10, 3961 NC Wijk bij Duurstede, The Netherlands).

Each of the 16 treatments was fed to 6 replicate pens of 20 broilers each (120 birds per treatment; total of 1,920 birds). One-day old male Ross 308 broiler chickens were sourced locally (Geflügelhof Möckern, Pabsdorfer Weg 9, 39291 Möckern, Germany). Twenty chickens were allocated to each of the 96 experimental floor pens in such a way as to ensure similar mean body weights across treatments. Birds were routinely vaccinated against Newcastle Disease and Gumboro on day 16. Pelleted feed as well as water were offered *ad libitum*. Automatic feeders were re-filled with pre-weighed amounts when required. During the first days of the starter phase, feed was additionally offered in flat plastic bowls. Initial bedding consisted of wood shavings, new straw bedding was added after three weeks about twice weekly. Lighting and temperature regimes followed breeder's recommendations and, where necessary (lighting), were adjusted to comply with local animal welfare regulations.

At the beginning of the experiment, all birds of each pen were weighed together. At the end of each of the three phases (days 10, 27, and 38) body weight (BW) was recorded on an individual animal basis as well as the feed remaining in the feeder. Feed efficiency (FE; g gain per kg feed) was calculated as the ratio of the increase (= gain) in BW and the amount of feed consumed (includes ingested and spilled material) during the respective phase. The increase in total animal production per phase was calculated as the sum of individual BW of birds (including losses) at the end of a fattening phase minus total BW at the beginning of that phase. The total losses (including removals) throughout the experiment were 3.18% (n = 61), with 5 losses (0.26%) being caused by the weighing procedures (distress and induced injuries). Losses did not correlate to methionine sources or inclusion levels.

On the last day of the feeding trial (day 38) those four birds of each pen (24 birds per treatment), that had a BW closest to the pen mean were selected. Selected birds were separated from non-selected birds and penned group-wise based upon their original treatment. Because slaughtering was not immediately possible, those birds received their respective experimental diet for further 36 h and were fastened about 12 h prior to slaughter. Birds were slaughtered in a commercial facility on day 40. After cooling of the carcass the breast including skin and bone was cut off. For each bird, residual carcass and breast were weighed together to determine carcass weight, and then breast was weighed individually. For further analysis, total carcass weight was put into relation to BW at day 38 on an individual animal basis (dressing ratio) and breast weight was put into relation to total carcass weight (breast-to-carcass-ratio; BCR).

Trial 2 was performed at the research facility of Fazenda do Glória of the Federal University of Uberlândia, Brazil. In this experiment 1,650 male Cobb 500 chickens were fed the experimental starter (days 1 to 14), grower (days 15 to 28) and finisher diets (days 29 to 42) mainly based on sorghum and soybean meal (Table 2). Whilst all essential amino acids were balanced according to an ideal protein concept (QuickChick, Version 1.0.0.12, Degussa GmbH, 2006), digestible Met+Cys to Lys was kept low with 6.7, 6.2 and 6.3 g/kg total Met+Cys in the basal starter, grower and finisher diet, respectively. Remaining nutrients and energy were formulated according to the Brazilian Tables for Poultry and Swine (Rostagno, 2005). Birds were fed either the unsupplemented basal diets or diets supplemented with five graded levels (0.03, 0.07, 0.12, 0.18, 0.25%) of either DL-Met or MHA-Ca. The calculated amino acid levels were confirmed by analysis. Each of the 11 treatments was fed to five replicate pens of 30 broilers each, summing up to 150 broilers per treatment. Birds were kept in floor pens covered with rice hulls. Temperature and lighting was in accordance with breeder's recommendations. Water and mash feed were offered for free consumption throughout the experiment. Birds were vaccinated against Gumboro Disease immediately post-hatch and on day 12. Feeders were re-filled with pre-weighed amounts when required.

Body weights as well as feed consumption were recorded at arrival at the research facility, at day 14, day 28 and at termination of the trial. Two birds per pen with body weights close to the pen average were selected for carcass evaluation including the determination of carcass yield and breast meat yield.

Statistical analysis and calculations

Recorded data were analyzed by an one-factorial

Table 2. Composition of the basal diets, calculated energy content and analysed crude protein and amino acid concentrations, Trial 2

Ingredients (g/kg)	Starter (days 0-14)	Grower (days 15-28)	Finisher (days 29-42)
Sorghum	581.15	656.74	666.41
Soybean meal (CP 48%)	350.18	273.26	253.47
Soybean oil	25.18	29.07	42.22
Starch	2.50	2.50	2.50
BioLys 65	5.52	5.18	4.76
L-threonine 98%	0.62	0.45	0.42
Premix microminerals	1.00	1.00	1.00
Premix vitamins	1.00	1.00	1.00
Limestone	9.14	8.78	8.21
Dicalcium phosphate (18%)	18.14	16.80	15.05
Salt	4.97	4.72	4.45
Choline chloride	0.60	0.50	0.50
AME_N (kcal/kg)	2,900	3,000	3,100
AME_N (MJ/kg)	(12.1)	(12.6)	(13.0)
Crude protein	245	228	212
Methionine	3.2	3.0	3.1
Met+Cys	6.7	6.2	6.3
Lysine	12.7	12.3	10.4
Threonine	8.8	8.1	8.2
Arginine	13.9	12.8	12.0

analysis of variance (ANOVA) taking account of the fixed effect of treatment. Treatment means were calculated as least square means, Tukey-Kramer test was applied to account for multiple comparisons between all treatments. Additionally, in experiment 1 for the parameter 'BW on day 38' in an attempt to quantitatively compare control and test Met sources (DL-Met65 and MHA-Ca, respectively) in relation to the reference material (pure DL-Met), the following approach was chosen: The performance of animals receiving the basal diet shall be regarded as the basal performance. Any change in the parameters of animal performance can be attributed to the increase in Met supplementation and shall, therefore, be regarded as the supplement-induced performance (SIP). Again, DL-Met shall be the reference substance. SIP of both, DL-Met65 and MHA-Ca, were put in relation to SIP of pure DL-Met. To assess the significance of the difference between pure DL-Met and DL-Met65 as well as MHA-Ca, paired comparisons (t-test) were performed. To account for multiple comparisons (5 supplemented levels, 2 comparisons per level), Bonferroni adjustment was applied to the level of significance.

Relative bio-availability as a measure of biological effectiveness was determined using a non-linear regression approach proposed by Littell et al. (1997). In short, non-linear response curves are fitted over the supplementation of the various (nutrient) sources with the assumptions of equal starting points and common plateau. RBV is then defined as the ratio of supplemented levels of test and standard (nutrient) source at a given response level (i.e. BW, gain, FE). In the present studies, pure DL-Met was used as the reference substance. DL-Met65 served as control and should obtain a RBV of 65%.

The model of determining the biological effectiveness is:

$$Y = B_1 + B_2 * \left(1 - e^{B_3*(X_1 + B_4*X_2 + B_5*X_3)}\right) + e$$

With

B_1	Common intercept
B_1+B_2	Common asymptote
B_3	Steepness coefficient for pure DL-Met
B_4	Biological effectiveness of MHA-Ca relativ to pure DL-Met (Steepness coefficient for MHA-Ca can be calculated from B4/B3)
B_5	Biological effectiveness of DL-Met65 relativ to pure DL-Met (only trial 1; Steepness coefficient for DL-Met65 can be calculated from B5/B3)
X_1	Supplementation (% in diet) of pure DL-Met
X_2	Supplementation (% in diet) of MHA-Ca
X_3	Supplementation (% in diet) of DL-Met65 (only trial 1)

Recently, Kratzer and Littell (2006) have questioned this approach. They claimed different locations for absorption and different metabolic pathways denying the assumption of a common asymptote. The authors of the present article do not agree with that position (see Discussion section), however, a preliminary analysis was performed according to the proposed nonlinear separate plateaus asymptotic regression approach (Kratzer and Littell, 2006). In experiment 1, no differences between the plateaus of DL-Met, MHA-Ca, and DL-Met65 could be found for main animal performance parameters BW and FE. Therefore, results in the present paper will be based on the nonlinear common plateau asymptotic regression as

Table 3. Average body weight (g) of male Ross 308 broilers fed graded levels of DL-Met, Met hydroxy analog calcium salt (MHA-Ca), or diluted DL-Met (65%; DL-Met65) in the course of Trial 1

Supplemental dietary addition		Compound %	BW day 1	BW day 10	BW day 27	BW day 38
1	Basal diet	0	41.6	187[a]	964[a]	1,725[a]
2	DL-Met	0.03	41.8	210[b,c]	1,255[c]	2,284[c]
3	DL-Met	0.06	41.8	230[d,e,f]	1,434[d]	2,592[d]
4	DL-Met	0.10	41.4	242[e,f,g]	1,507[d,e,f]	2,702[d,e]
5	DL-Met	0.15	41.7	249[g]	1,590[f]	2,813[e,f]
6	DL-Met	0.21	41.6	251[g]	1,592[f]	2,854[f]
7	MHA-Ca	0.03	41.4	206[b,c]	1,160[b]	2,117[b]
8	MHA-Ca	0.06	41.9	222[c,d]	1,325[c]	2,397[c]
9	MHA-Ca	0.10	41.1	227[d,e]	1,441[d,e]	2,580[d]
10	MHA-Ca	0.15	41.7	246[g]	1,577[f]	2,766[e,f]
11	MHA-Ca	0.21	41.9	248[g]	1,581[f]	2,788[e,f]
12	DL-Met65	0.03	41.6	205[b]	1,147[b]	2,112[b]
13	DL-Met65	0.06	41.7	219[b,c,d]	1,295[c]	2,351[c]
14	DL-Met65	0.10	41.5	227[d,e]	1,438[d,e]	2,567[d]
15	DL-Met65	0.15	41.1	243[f,g]	1,529[e,f]	2,736[e,f]
16	DL-Met65	0.21	41.3	246[f,g]	1,567[f]	2,781[e,f]
Pooled SEM			0.27	3.2	18.1	28.5

Values not sharing at least one superscript are statistically ($p<0.05$) different from each other.

described above. Findings by Sauer et al. (2007) who performed a meta-analysis on the RBV of methionine sources based on 40 data sets support the assumption of common plateau.

All regression analyses were performed on individual replicate data and not on treatment means.

All data analyses were conducted using the software package SAS/STAT (SAS Institute, 2004). If not stated otherwise, the level of significance was chosen at $p<0.05$ (type I experiment-wise error rate).

RESULTS

Trial 1 (Feedtest, Germany)

Between the 96 experimental pens mean BW of birds on day 1 was 41.55 g, ranging from 40.30 to 42.85 g and did not differ ($p = 0.68$) between treatments (Table 3).

In all three phases of the experiment, there was an effect of Met-source supplementation on BW gain and consequently on BW (Table 3). The unsupplemented basal diet (treatment 1) consistently had the lowest BW (187, 964, and 1725 g for days 10, 27, and 38, respectively). An increase in Met supply by either source led to increased BW. Treatments 5 and 6 (pure DL-Met, fourth and fifth level of supplementation) had regularly the highest BW (250, 1590 and ~2,830 g for days 10, 27, and 38, respectively). Feed consumption was lowest in the control group (treatment 1) throughout the experiment. Averaged over the whole trial, daily feed consumption per bird ranged from about 98 to 125 g (treatments 1 and 5, respectively; Table 4). Similar to BW and BW gain, there was an effect of level of supplement on FC within the source of methionine investigated. However, this effect did not seem as strong as in the parameters BW and BW gain. Increasing supplementation increased feed efficiency in either Met source (Table 4) from 526 g/kg (Basal diet) to 595, 581, and 582 g/kg (DL-Met, MHA-Ca, and DL-Met65, respectively, supplemented at 0.21% of diet).

The dressing percentage ranged from 67 to 72% (treatments 1 and 16, respectively; Table 4). With increasing Met supplementation not only the proportion of the carcass (in relation to BW) increased, but also the proportion of

Table 4. Feed consumption (FC), feed efficiency (FE) and carcass data of 38-d-old male Ross 308 broilers fed graded levels of DL-Met, Met hydroxy analog calcium salt (MHA-Ca), or diluted DL-Met (65%; DL-Met65), Trial 1

Supplemental dietary addition		Compound %	FC (g/d)	FE (g gain per kg feed)	Dressing ratio[1] (%)	Breast[2]-to-carcass-ratio (%)
1	Basal diet	0	82.0^{a}	526^{a}	67.2^{a}	29.1^{a}
2	DL-Met	0.03	$105.3^{b,c,d}$	$544^{a,b,c,d}$	$68.0^{a,b}$	$32.1^{b,c}$
3	DL-Met	0.06	$116.9^{e,f}$	$557^{b,c,d,e,f}$	$70.1^{b,c,d}$	$33.0^{b,c,d,e}$
4	DL-Met	0.10	119.1^{f}	$570^{c,d,e,f,g}$	71.2^{d}	$34.8^{e,f,g}$
5	DL-Met	0.15	120.9^{f}	$586^{f,g}$	71.5^{d}	$35.3^{f,g}$
6	DL-Met	0.21	120.9^{f}	595^{g}	71.1^{d}	36.0^{g}
7	MHA-Ca	0.03	97.8^{b}	$542^{a,b,c}$	$68.6^{a,b,c}$	31.3^{b}
8	MHA-Ca	0.06	$110.2^{d,e}$	$546^{a,b,c,d}$	$70.0^{b,c,d}$	$32.9^{b,c,d,e}$
9	MHA-Ca	0.10	$117.0^{e,f}$	$555^{b,c,d,e}$	$70.2^{b,c,d}$	$33.0^{b,c,d,e}$
10	MHA-Ca	0.15	123.2^{f}	$566^{c,d,e,f,g}$	$70.4^{b,c,d}$	$33.8^{c,d,e,f}$
11	MHA-Ca	0.21	121.0^{f}	$581^{e,f,g}$	71.2^{d}	$34.4^{d,e,f,g}$
12	DL-Met65	0.03	$99.5^{b,c}$	$531^{a,b}$	$68.0^{a,b}$	31.5^{b}
13	DL-Met65	0.06	$108.1^{c,d}$	$546^{a,b,c,d}$	$69.9^{b,c,d}$	$32.6^{b,c,d}$
14	DL-Met65	0.10	$116.9^{e,f}$	$553^{a,b,c,d,e}$	$69.8^{b,c,d}$	$33.1^{b,c,d,e}$
15	DL-Met65	0.15	120.2^{f}	$573^{d,e,f,g}$	$71.0^{c,d}$	$35.1^{f,g}$
16	DL-Met65	0.21	120.3^{f}	$582^{e,f,g}$	72.0^{d}	$34.4^{d,e,f,g}$
Pooled SEM			1.73	5.9	0.50	0.40

Values not sharing at least one superscript are statistically ($p<0.05$) different from each other.

[1] The feeding study ended on day 38, animals selected for carcass evaluation were fed another 36 h with the respective diets and slaughtered on day 40.

[2] Breast including bone and skin.

Table 5. Supplement induced performance (SIP) for BW on day 38, absolute for DL-Met and relative to that for Met hydroxy analog calcium salt (MHA-Ca), and diluted DL-Met (65%; DL-Met65) in Trial 1

	Inclusion of Met source (% in diet)					
	0	0.03	0.06	0.10	0.15	0.21
Basal diet (g)	1,725					
DL-Met: SIP absolute (g)		559	867	977	1,088	1,129
MHA-Ca: SIP relative to DL-Met		0.70*	0.78*	0.88*	0.96	0.94
DL-Met65: SIP relative to DL-Met		0.69*	0.72*	0.86*	0.93	0.94

* SIP of MHA-Ca and DL-Met65 significantly lower than that of DL-Met (accounting for multiple paired comparisons by Bonferroni-adjustment).

breast in relation to the carcass increased from 29 to 36% (treatments 1 and 6, respectively; Table 4).

The supplement-induced performance (SIP) describes that part of the respective parameters caused by the supplementation, i.e. the difference between basal diet and treatment groups. For final BW (day 38), SIP of DL-Met65 and MHA-Ca relative to that of DL-Met ranged from 0.69 to 0.94 and from 0.70 to 0.96, respectively (Table 5). As can be seen, with increasing supplementation levels the gap between pure DL-Met and the other two Met sources decreased. For both DL-Met65 as well as MHA-Ca, SIP was significantly lower than that of pure DL-Met for the first three supplementation levels (0.3, 0.6, and 1.0 g/kg). Similar observations were made in other parameters of animal performance (results not shown).

If the clearly curvilinear relationship between Met supplementation and BW (Figure 1) was analyzed by multi-exponential common plateau regression, MHA-Ca and DL-Met65 yielded a RBV of 65 and 61%, respectively, for final BW (Table 6). The RBV for FE varied somewhat depending upon the period analyzed. Data derived from the Starter phase (days 1-10) only were difficult to analyze, the standard error of estimation being high. However, for the whole experiment (days 1-38), RBV for MHA-Ca and DL-Met65 was 60 and 62%, respectively. The RBV for BCR were 53 and 63 for MHA-Ca and DL-Met65, respectively (Table 6).

For all parameters analyzed (with the exception of FE days 1-10 and dressing ratio) RBV for MHA-Ca was significantly lower than 84%, i.e. the figure which would have been expected from its chemical properties (Table 6).

Trial 2 (Federal University Uberlândia)

Supplementation of either DL-Met or MHA-Ca produced significant responses on body weight gain and feed efficiency in all phases (Table 7) whereas feed intake

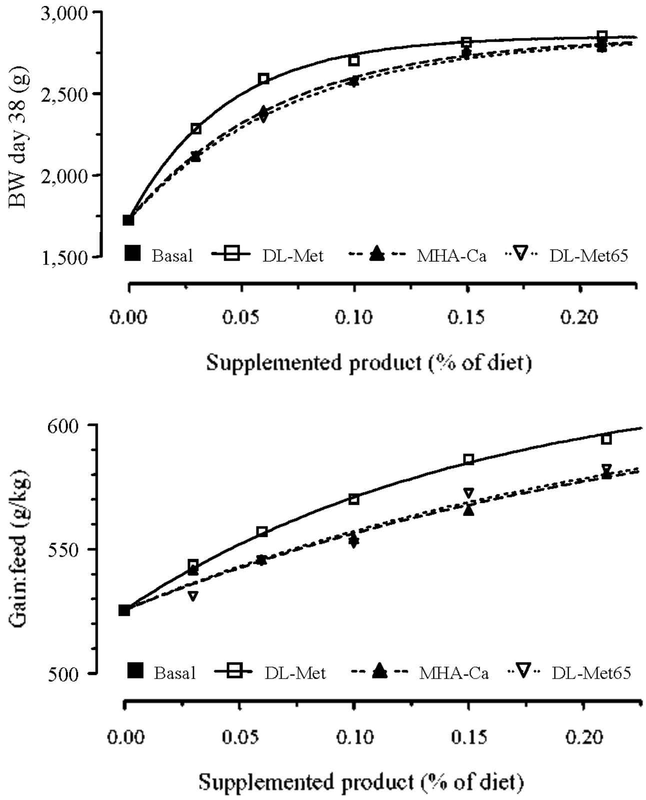

Figure 1. Curvilinear increase of body weight and decrease of feed conversion ratio with increasing supplementation of DL-Met, Met hydroxy analog calcium salt (MHA-Ca), and diluted DL-Met (65%; DL-Met65) (Experiment 1).

was not affected from 1 to 28 and 1 to 42 days of age (data not shown). Recorded performance was in agreement with breeder's recommendation. Carcass yield was also significantly improved by both Met sources whereas breast meat yield showed a significant effect only for the highest DL-Met supplementation as compared to the control. Overall viability ranged between 92 and 95% and was not

Table 6. Estimated biological effectivnesses of Met hydroxy analog calcium salt (MHA-Ca) and diluted DL-Met (65%; DL-Met65) in relation to pure DL-Met, Trial 1

Parameter [1]	MHA-Ca			DL-Met65		
	Estimate	s.e.	95% confidence interval	Estimate	s.e.	95% confidence interval
BW day 10	70*	7.1	56-84	64	6.5	51 to 77
BW day 27	69*	4.4	61-78	63	3.9	55 to 70
BW day 38	65*	3.8	57-72	61	3.6	54 to 68
BWgain days 1-10	70*	7.0	56-84	64	6.4	52 to 77
BWgain days 1-27	69*	4.4	61-78	63	3.9	55 to 70
BWgain days 1-38	65*	3.8	58-73	61	3.5	54 to 68
FE days 1-10	71	19.0	34-109	60	15.6	29 to 90
FE days 1-27	59*	7.7	44-74	61	7.8	45 to 76
FE days 1-38	60*	8.7	43-78	63	8.9	45 to 81
Carcass weight	67*	4.8	57-76	59	4.3	51 to 68
Breast weight	63*	4.7	53-72	60	4.5	52 to 69
BCR	53*	8.0	37-69	63	9.5	44 to 82

[1] BW = Body weight; BW gain = Daily body weight gain; FE = Feed efficiency; BCR = Breast-to-carcass ratio.

* Estimated biological effectiveness is significantly lower than the 84% which would be expected from the MHA-Ca's chemical composition.

Table 7. Feed intake, body weight and feed efficiency of male Cobb 500 chickens fed graded levels of DL-Met and Met hydroxy analog calcium salt (MHA-Ca) supplemented to a Met+Cys-deficient basal diet, Trial 2

Product	Inclusion level (%)	Body weight (g)			FE (g gain per kg feed)			Viability (%)	Dressing ratio (%)	Breast[1]-to-carcass-ratio (%)
		Day 14	Day 28	Day 42	Days 0-14	Days 0-28	Days 0-42	Days 0-42		
Control	0	376 [a]	1,273 [a]	2,485 [a]	610 [a]	579 [a]	523 [a]	93.1	72.8 [a]	33.2 [a]
DL-Met	0.03	417 [bc]	1,364 [bc]	2,649 [b]	669 [ab]	620 [bc]	545 [b]	92.0	74.9 [ab]	34.8 [ab]
DL-Met	0.07	442 [cd]	1,398 [cd]	2,624 [b]	682 [b]	635 [bcd]	551 [b]	94.8	75.7 [b]	34.2 [ab]
DL-Met	0.12	457 [d]	1,449 [d]	2,708 [b]	684 [b]	633 [bcd]	555 [b]	92.1	75.2 [ab]	35.3 [ab]
DL-Met	0.18	449 [d]	1,442 [cd]	2,655 [b]	673 [ab]	648 [cd]	555 [b]	94.1	74.3 [ab]	35.0 [ab]
DL-Met	0.25	439 [cd]	1,460 [d]	2,669 [b]	680 [b]	653 [d]	558 [b]	91.7	76.1 [b]	35.9 [b]
MHA-Ca	0.03	386 [ab]	1,313 [ab]	2,593 [ab]	638 [ab]	609 [b]	540 [ab]	93.9	74.5 [ab]	33.5 [ab]
MHA-Ca	0.07	427 [cd]	1,365 [bc]	2,613 [ab]	671 [ab]	620 [bc]	548 [b]	94.2	73.8 [ab]	34.0 [ab]
MHA-Ca	0.12	441 [cd]	1,421 [cd]	2,650 [b]	678 [b]	641 [cd]	550 [b]	93.3	74.5 [ab]	33.7 [ab]
MHA-Ca	0.18	448 [cd]	1,450 [d]	2,644 [b]	674 [ab]	640 [cd]	547 [b]	92.1	75.5 [b]	34.5 [ab]
MHA-Ca	0.25	448 [cd]	1,468 [d]	2,679 [b]	679 [b]	660 [d]	553 [b]	92.0	75.7 [b]	35.2 [ab]

Values not sharing at least one superscript are statistically ($p<0.05$) different from each other.

[1] Breast including bone.

attributable to dietary treatments. Majority of losses occurred in the finisher phase.

For all parameters investigated the estimated RBV was below 84% (Table 8), ranging from 30 to 71%. Because of high error of estimation, only RBV for BW on day 14 and for BW on day 42 were significantly lower than 84% whilst for BW at day 28 or breast weight RBV was close to be significant. The high error of estimation could at least partially be explained by the low level of response in combination with its relatively high variability between increasing levels of supplementation. Whilst in experiment 2 overall weight gain increased by maximal 9% (control vs. 0.12% DL-Met, Table 7), weight gain increased by approximately 60% to 65% in trial 1. The low level of response in combination with a relative poor fit (R^2 = 0.86) of the data points to the curves might explain the very low RBV of 30% determined for overall weight gain to a certain extent (Table 8). For days 0 to 42 FE data fitted better and the response was strong, resulting in a RBV of 59%. In contrast to breast weight (Figure 2) responses of breast meat yield (breast-to-carcass ratio) did not indicate an asymptote and thus multi linear regression analysis (Littell et al., 1997) was applied revealing a RBV of 55%.

Table 8. Estimated biological effectivnesses of Met hydroxy analog calcium salt (MHA-Ca) in relation to pure DL-Met, Trial 2 (nonlinear common plateau asymptotic regression)

Parameter[1]	Estimate	s.e.	95% confidence interval
BW day 14	47*	11.3	24 to 69
BW day 28	64	12.4	39 to 89
BW day 42	30*	21.5	-13 to 73
FE days 1-14	36	26.2	-17 to 88
FE days 1-28	71	15.9	39 to 103
FE days 1-42	59	25.2	8 to 109
Carcass weight	64	20.0	24 to 105
Breast weight	55	15.8	23 to 86
BCR linear[2]	55	20.6	13 to 96

[1] BW = Body weight; FE = Feed efficiency; BCR = Breast-to-carcass ratio.

[2] Linear slope-ratio model had to be applied due to linear response of BCR over the studied range of Met source supplementation.

* Estimated biological effectiveness is significantly lower than the 84% which would be expected from the MHA-Ca's chemical composition.

DISCUSSION

The example of final BW in trial 1 (Table 5) demonstrated, that an 'effectiveness' of one Met source in relation to another is impossible to derive from individual supplementation levels. The response to DL-Met65 relative to that of pure DL-Met increased from 0.69 to 0.94 with increasing supplementation levels (Table 5). The reason for this is the underlying law of diminishing returns. Similar observations have been made by Hoehler (2006) on data of Lemme et al. (2002), emphasizing 'relative responses' being inappropriate as a tool to compare various nutrient (Met) sources.

In trial 1, animal performance of MHA-Ca supplemented birds was similar to that of DL-Met65

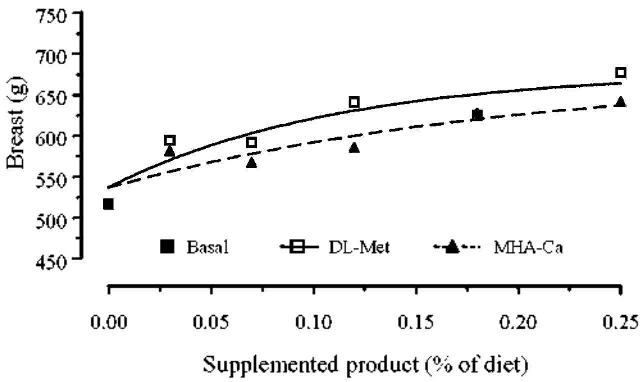

Figure 2. Curvilinear increase of breast meat yield with increasing supplementation of DL-Met and Met hydroxy analog calcium salt (MHA-Ca) (Experiment 2).

supplemented birds (Tables 3 and 4), indicating that both supplements released a similar methionine activity to the bird's metabolism at the corresponding inclusion levels. By definition, DL-Met65 has a RBV of about 65% relative to that of pure DL-Met. Consequently, the relative availability of MHA-Ca should be similar or only slightly above this value. This approach is unsatisfying, because in each study one would need to have not only the reference material and the test material but also the reference material diluted to one or more levels in the range of the expected efficacy of the test substance. All of which is time and cost consuming and requires a lot more birds for the study than if a non-linear regression approach was applied (Figure 1).

Simultaneous nonlinear common plateau asymptotic regression has been proposed as the appropriate statistical analysis to obtain RBV (Littell et al., 1997). Recently, Kratzer and Littell (2006) questioned this approach for the comparison of Met sources because of different locations for absorption and different metabolic pathways of the various Met sources. They supported their position by a survey of several studies comparing the effect of MHA-FA and DL-Met on BW gain. A separate analysis of the respective curves gave an indication that plateaus might be different between the two Met sources. In the present study 1, this assertion of Kratzer and Littell (2006) was tested and had to be negated: the common plateaus for final BW and FE were 2852 g (s.e. 17.4) and 621 g/kg (s.e. 24.9). When estimated separately, the plateaus for DL-Met, MHA-Ca, and DL-Met65 were 2,848±22.0, 2,858±33.9, and 2,857±36.9 g, and 615±21.1, 635±121.7, and 749±368.7 g/kg, respectively, and did not differ significantly. Not only did the calculations of Kratzer and Littell (2006) contain some statistical obscurities as pointed out by Piepho (2006), but they have changed the perspective: Whereas the biological effectiveness or relative bioavailability proposed by Littell et al. (1997) was based on the ratio of supplementation levels at a given response level (in the case of nonlinear common plateau asymptotic regression this ratio is constant), Kratzer and Littell (2006) suggested contrastingly the use of relative response, which is defined as the ratio of total or supplement induced responses at a given supplementation level. This change of perspective appears to be misleading because it uses the same terminology as in the determination of RBV (e.g. 'relative efficiency' is synonymously used for RBV (Littell et al., 1997) and relative response (Kratzer and Littell, 2006), and, furthermore, may yield illogical results (Hoehler, 2006). Even if there were reasons to assume separate plateaus for the different nutrient sources, biological effectiveness should be determined as the ratio of supplementation levels at a given response level. Sauer et al. (2007) in their meta-analysis including data out of 40 experiments from peer reviewed publications on methionine source comparisons concluded that a common plateau can be assumed.

Diluted DL-Met (DL-Met65) was included in trial 1 as an internal standard. RBV of DL-Met65 reflected the degree of dilution, i.e. approximately 65%. RBV for final BW, average BW gain, average FE and breast-to-carcass ratio were only slightly lower than expected, ranging from 61 to 63% (Table 6). Also for sub-periods of the experiment, similar RBV were obtained. Judging the overall results for DL-Met65, the selected nonlinear model could be regarded as validated and an appropriate approach to determine RBV of methionine sources.

Although the present studies were conducted with two different broiler genetics (Ross 308, Cobb 500), under different environmental conditions (Central Europe, Brazil) and with completely different main dietary ingredients (wheat, sorghum), the results regarding RBV of MHA-Ca were very similar: In experiment 1, RBV of MHA-Ca for animal performance parameters over the whole rearing period (days 1-38) varied from 60 to 65% (Table 6). RBV for carcass and breast weight were in a similar range, but RBV for dressing ratio and breast-to-carcass ratio varied slightly more (Table 6). The RBV for the dressing ratio (79%) was about as high as would have been expected from the chemical properties of MHA-Ca (84% active substance content); however, not only did it coincide with a high standard error (Table 6) but the RBV for DL-Met65 was on a similar level (78%). It might well be, that the proportion of carcass weight from body weight does not respond as sensitive as other parameters of animal performance.

In contrast to trial 1, the magnitude of the responses for weight gain and feed conversion was weaker in trial 2. Nevertheless, although RBV values were in part unexpectedly low, also this study suggested a superiority of DL-Met over MHA-Ca in the parameters tested. Determined RBV were maximum 71% (FE at 1-28 days of life) and thus in the range of the results of trial 1. Moreover, in the literature survey on efficiency of liquid MHA-FA by Lemme and Petri (2003), RBV decreased from about 65% to 62% to 59% for weight gain (n = 45), feed conversion (n = 41), and breast meat yield (n = 5), respectively - a ranking which seems to be similar for MHA-Ca in the study presented here. The literature survey on efficiency of MHA-Ca by Lemme (2004) did not contain data on breast meat yield but RBV for feed conversion was lower (about 62%, n = 32) than for weight gain (65%, n = 33). Averaging RBV of the studies presented in this paper determined for overall weight gain (except for experiment 2), feed efficiency and breast meat yield revealed values of about 66%, 64%, and 54%, respectively, with an overall mean of 63%. This confirms - at least when compared to Lemme and Petri (2003) and Lemme (2004) - that bioefficiency of MHA-Ca has not changed over the years and is not different from that of liquid MHA-FA (Mandal et al., 2004) when compared on "as is" basis. As early as in 1984, Potter et al. reviewed the literature on this topic (n = 30, including 6 turkey assays) and concluded that MHA-Ca efficiency is significantly inferior to DL-Met with an average RBV of 71% (only broiler assays) on "as-is" basis.

The reasons behind the lower RBV of MHA-Ca compared with DL-Methionine may be explained by the studies reported in the following: After feeding radiolabelled (^{14}C) MHA-Ca and DL-Met, Lingens and Molnar (1996) recovered only 4.4% of the ingested ^{14}C in the excreta of DL-Met fed broilers but 17% in the excreta of MHA-Ca fed broilers. This finding indicated a less effective absorption of MHA-Ca as compared to DL-Met. In addition, the ^{14}C recovered in breast and leg muscle was higher in DL-Met fed than in MHA-Ca fed broilers suggesting a relationship between availability of dietary methionine sources and their incorporation into the muscle tissue. Esteve-Garcia (1988) and Esteve-Garcia and Austic (1993) also reported a higher excretion of radiolabelled MHA-Ca compared to radiolabelled DL-Met. In four subsequent trials the percentage of unabsorbed MHA-Ca was 5.5-fold higher than that of DL-Met. A similar experiment using ^{3}H labelled MHA-FA and DL-Met further suggested microbial degradation in the small intestine to be a major reason for the low RBV of hydroxy analogues of methionine.

CONCLUSIONS

A controversy exists as of how to determine biological effectiveness of one Met source in relation to another. In 1997, Littell et al. proposed the concept of relative bioavailability (RBV), which in a nonlinear common plateau asymptotic regression relates the respective levels of supplementation at a given response. Our studies have validated once again this approach and supported previous statements that the contrasting concept of "relative responses" is a misleading and inconclusive measure of efficiency.

Graded supplementation of DL-Met and MHA-Ca to Met+Cys deficient basal diets resulted in significant performance improvements of different magnitude in two feeding studies. Across the studies with several broiler genetics and differing basal diet composition, estimated RBV of MHA-Ca in relation to pure DL-Met averaged to 63% and was in many cases significantly lower than 84% which is the MHA content in MHA-Ca. Whereas the type of diet obviously influenced the magnitude of the responses, RBV was not affected. The set up of trial 1 - including also graded levels of DL-Met65 with a purity of 65% DL-Met - confirmed multi-exponential common plateau regression to be a suitable method to determine the relative bioavailability of methionine sources.

REFERENCES

Drew, M. D., A. G. van Kessel and D. D. Maenz. 2003. Absorption of methionine and 2-hydroxy-4-methylthiobutanoic acid in conventional and germ-free chickens. Poult. Sci. 82:1149-1153.

Esteve-Garcia, E. 1988. Digestion and excretion of methionine sources for poultry. PhD thesis, Cornell University, Ithaca, New York, USA.

Esteve-Garcia, E. and R. E. Austic. 1993. Intestinal absorption and renal excretion of dietary methionine sources by the growing chicken. J. Nutr. Biochem. 4:576-587.

Hoehler, D. 2006. Letter to the editor: No evidence for different dose responses of commercial methionine sources in broilers. Poult. Sci. 85:2047.

Hoehler, D. 2007. Letter to the editor (Letter): A misleading approach to determine the methionine activity of organic trace minerals. Poult. Sci. 86:1611-1612.

Jansman, A. J. M., C. A. Kan and J. Wiebenga. 2003. Comparison of the biological efficacy of DL-methionine and hydroxy-4-methylthiobutanoic acid (HMB) in pigs and poultry. CVB documentation report Nr. 29, Centraal Veevoederbureau, Lelystad, The Netherlands.

Kratzer, D. D. and R. C. Littell. 2006. Appropriate statistical methods to compare dose responses of methionine sources. Poult. Sci. 85:947-954.

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. Poult. Sci. 81:838-845.

Lemme, A. and A. Petri. 2003. The effectiveness of liquid methionine hydroxy analogue relative to DL-methionine - A literature review. AminoNews™ 4(3):1-10.

Lemme, A. 2004. Relative effectiveness of the methionine hydroxy analogue calcium salt in broilers and layers. AminoNews™ 5(3):7-12.

Lingens, G. and S. Molnar. 1996. Studies on metabolism of broilers by using 14C-labelled DL-Methionine and DL-Methionine hydroxy analogue Ca-salt. Arch. Anim. Nutr. 49:113-124.

Littell, R. A., P. R. Henry, A. J. Lewis and C. B. Ammermann. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672-2683.

Mandal, A. B., A. V. Elangovan and T. S. Johri. 2004. Comparing bio-efficacy of liquid DL-methionine hydroxy analogue free acid with DL-methionine in broiler chickens. Asian-Aust. J. Anim. Sci. 17:102-108.

Piepho, H.-P. 2006. Letter to the editor: A cautionary note on appropriate statistical methods to compare dose responses of methionine sources. Poult. Sci. 85:1511-1512.

Potter, L. M., G. P. Schmidt, M. E. Blair, J. R. Shelton and B. A. Bliss. 1984. MHAC versus DL-methionine-Part I: The methionine controversy. Animal Nutrition and Health (March-April):14-17.

Richards, J. D., J. J. Dibner and C. D. Knight. 2007. Letters to the editor (Reply): DL-2-hydroxy-4-(methylthio)butanoic acid from any commercial source is fully available as a source of methionine activity. Poult. Sci. 86:1613-1614.

SAS Institute. 2004. SAS/STAT User's Guide. Release 9.1. SAS Institute Inc., Cary, NC.

Sauer, N., K. Emrich, H. P. Piepho, A. Lemme, M. S. Redshaw and R. Mosenthin. 2007. Meta-analysis on the relative efficiency of methionine-hydroxy-analogue-free-acid compared with DL-methionine in broilers using non-linear mixed models. Poult. Sci. (In press). (partly published in: Sauer, N., K. Emrich, H. P. Piepho, A. Lemme, M. S. Redshaw and R. Mosenthin. 2007. Meta-analysis on the relative efficiency of the liquid hydroxy analogue compared with DL-methionine in broilers using multi-exponential regression. 16th European Symposium on Poultry Nutrition, August 27-30, Strasbourg, France: 4).

Vázquez-Añon, M., D. Kratzer, R. Gonzélez-Esquerra, I. G. Yi and C. D. Knight. 2006. A multiple regression model approach to contrast the performance of 2-hydroxy-4-methylthio butanoic acid and DL-methionine supplementation tested in broiler experiments and reported in the literature. Poult. Sci. 85:693-705.

Yi, G. F., C. A. Atwell, J. A. Hume, J. J. Dibner, C. D. Knight and J. D. Richards. 2007. Determining the methionine activity of Mintrex organic trace minerals in broiler chicks by using radiolabel tracing or growth assay. Poult. Sci. 86:877-887.