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BIOAVAILABILITY OF LIQUID METHIONINE HYDROXY ANALOGUE-FREE ACID RELATIVE TO DL-METHIONINE IN BROILERS

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Abstract

ZELENKA JIŘÍ, HEGER JAROSLAV, MACHANDER VLASTISLAV, WILTAFSKY MARKUS, LEŠŤÁK MARTIN: *Bioavailability of liquid methionine hydroxy analogue-free acid relative to DL-methionine in broilers.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 5, pp. 1513–1520

An experiment with broiler chickens was conducted to compare the relative bioavailability of liquid methionine hydroxy analogue free acid (MHA-FA) with that of DL-methionine (DLM) during fattening to 35 days of age. Ross 308 male chicks were allotted to 9 treatments, each consisting of six replicates of 140 birds/pen. Four graded levels (0.04, 0.08, 0.16, and 0.28%) of MHA-FA or DLM products (weight/weight comparison) were added to a maize-wheat-soyabean meal basal diet deficient in sulphur amino acids. The criteria of response were body weight, feed conversion ratio, carcass yield and breast meat yield. Significant responses to graded levels of both methionine sources were observed in all response criteria. Using a multi-exponential model describing the dose-response relationships, the bioavailability estimates of MHA-FA relative to DLM on a weight-to-weight basis were 68, 70, 54 and 59% for body weight, feed conversion, carcass yield and breast meat yield, respectively. If MHA-FA was compared with DLM on equimolar basis its bioavailability was 77.7, 79.0, 59.3 and 64.6 for body weight, feed conversion, carcass yield and breast meat yield, respectively. The bioavailability of MHA-FA for carcass yield and breast meat yield was significantly ($P < 0.05$) lower than that of DLM on a weight-to-weight and on equimolar basis.

DLM, MHA-FA, chicken, weight gain, feed conversion, carcass yield, breast meat yield

Unlike mammals, broiler chickens require larger quantities of sulphur amino acids (SAA) to maximize their performance. Besides their various functions in metabolism, the high requirements for both methionine and cysteine are attributed to feather formation during growth. The concentration of total sulphur amino acids in feather protein is about 2.5 times higher than that of feather-free carcass (Stilborn *et al.*, 1997, 2010). Furthermore, the average cysteine to methionine ratio in feather protein was found to be 7-13:1 (Stilborn *et al.*, 1997) and cysteine was thus considered to be the first-limiting amino acid in most poultry diets (Baker, 1976). For practical reasons, however, the diets are commonly supplemented with DL-methionine (DLM) or its hydroxy analogue, i.e. DL-2-hydroxy-4(methylthio)butanoic acid (methionine hydroxy analogue free acid, MHA-FA).

Even though a large number of studies comparing the bioavailability of DLM and MHA-FA in broilers have been carried out during the last 40 years, there is still a considerable controversy regarding this question. Several authors (Waldroup *et al.*, 1981; Liu *et al.*, 2006; Vázquez-Añón *et al.*, 2006) found that both sources of methionine activity were equivalent on equimolar basis in promoting chicken growth. In contrast, the results of other studies (Huyghebaert, 1993; Lemme *et al.*, 2002; Payne *et al.*, 2006; Dilger and Baker, 2008) indicated that MHA-FA was substantially less biologically efficient than DLM. There are many factors which may be responsible for the inconsistent results described in the literature,

including the composition of basal diets, range of methionine source supplements, SAA levels relative to the requirement or methionine to cysteine ratio as well as their levels relative to other essential AA. The selection of a proper method of experimental data interpretation may also play a significant role.

The aim of the present experiment was to determine the biological effectiveness of MHA-FA relative to DLM added to practical-type broiler diets in graded amounts, using growth performance and carcass quality as the criteria of response.

MATERIAL AND METHODS

The experiment was conducted at the International Poultry Testing Station Ústrašice. The animal procedures were reviewed and approved by the Animal Care Committee of the Mendel University in Brno. A total of 7560 day-old male Ross 308 broiler chicks were randomly assigned to 9 treatments in such a way as to ensure similar mean body weights across treatments. There were six replicates per treatment (140 chicks per pen). Chickens were kept in the windowless house with full climatic control, on deep litter from wood shavings. Each pen was equipped with manually filled tube feeders and nipple drinkers. The stocking density was 17 broilers per square meter. Heating and lighting programmes were in accordance with Ross Broiler Management Manual (2009). On days 10, 24 and 35, the chickens were weighed individually. At the same time, feed consumption per pen was recorded. On the last day of experiment, five birds of each pen having body weights closest to the pen mean were selected, slaughtered and carcass and breast meat (boneless and without skin) yields were determined.

Basal starter (d 1 to 10), grower (d 11 to 24) and finisher (d 25 to 35) diets were formulated to be deficient in sulphur amino acids (approximately 60% of levels recommended by Ross Nutrition Supplement, 2009). All other nutrients and energy met or exceeded Ross Nutrition Supplement, 2009 – Tab. I). To the basal diets, DLM or MHA-FA were added at four levels (0.04, 0.08, 0.16, and 0.28%) on a product (weight-to-weight) basis, thus forming nine experimental diets for each phase of growth (Tab. II). In order to optimize homogenous distribution in the feeds, the supplements of

II: *Experimental design and supplemental levels of methionine sources*

Treatment	Methionine source supplements (%)	
	DLM[1)]	MHA-FA[2)]
Basal	-	-
DLM1	0.040	-
DLM2	0.080	-
DLM3	0.160	-
DLM4	0.280	-
MHA1	-	0.040
MHA2	-	0.080
MHA3	-	0.160
MHA4	-	0.280

[1)] DL-Methionine, purity 99%
[2)] Methionine hydroxy analogue free acid, 88% of active substance

I: *Composition of basal diets and analyzed nutrient contents (%, air-dry basis)*

	Starter 1–10 d	Grower 11–24 d	Finisher 25–35 d
Maize	32.00	35.00	38.00
Wheat	22.29	24.13	26.08
Soyabean meal, 48% CP	36.88	31.56	26.93
Soyabean oil	4.81	5.84	5.81
Dicalcium phosphate	1.81	1.6	1.49
Calcium carbonate	1.11	0.9	0.88
Salt	0.28	0.28	0.27
Sodium bicarbonate	0.13	0.13	0.14
L-Lysine.HCl	0.19	0.14	0.12
L-Threonine	0.07	0.05	0.03
L-Valine	0.05	0.02	-
Vitamin and micromineral premix	0.38	0.35	0.25
AME_n MJ/kg [1)]	12.67	13.19	13.40
Crude protein	22.92	20.75	18.60
Methionine	0.32	0.31	0.27
Cysteine	0.37	0.35	0.32
Lysine	1.35	1.18	1.00
Threonine	0.89	0.82	0.71

[1)] Calculated

MHA-FA containing 88% active substance and 12% water were added to the diets in form of a dry premix using silica powder as a premix carrier. During the experiment, the chickens were allowed free access to pelleted diets and water.

The diets were analyzed for nitrogen using Dumas procedure and for protein-bound and free amino acids by ion-exchange chromatography as described by Llames and Fontaine (1994) (Tab. I). Supplemented liquid MHA-FA was analyzed using the method of Naumann *et al.* (1997).

The performance data were analyzed as completely randomized block design using analysis of variance procedures. When significant value for treatment effect ($P < 0.05$) was observed, the differences between means were assessed using Tukey HSD test. Floor pen was the experimental unit for all analyses. To estimate the biological availability of MHA-FA relative to DLM, a multiexponential model proposed by Noll *et al.* (1984) and Littell *et al.* (1997) was used:

$$y = a + b\,(1 - e^{(c_1x_1 + c_2x_2)}),$$

where
y.....performance criterion
a .....intercept (performance with the basal diet)
b.....asymptotic response
$a + b$.....common asymptote (maximum performance level)
c_1.....steepness coefficient for DLM
c_2.....steepness coefficient for liquid MHA-FA
x_1.....dietary level of DLM
x_2.....dietary level of liquid MHA-FA.

The relative bioavailability value (RBV) for liquid MHA-FA was defined as the ratio of steepness coefficients c_2/c_1. To calculate the confidence intervals for the c_2/c_1 ratio, the model was reparametrized yielding the following equation:

$$y = a + b\,(1 - e^{c_1(x_1 + x_2c_2/c_1)}).$$

III: *Mean body weights of chickens (g)*

Treatment	Days of age			
	1	10	24	35
Basal	40.5^{a}	217^{a}	899^{a}	1 633^{a}
DLM1	40.5^{a}	258^{bc}	1 088^{bc}	1 964^{b}
DLM2	40.5^{a}	273^{bcde}	1 232^{def}	2 164^{cd}
DLM3	40.8^{a}	276^{bcde}	1 243^{def}	2 220^{cd}
DLM4	40.8^{a}	303de	1 323^{f}	2 265^{d}
MHA1	40.5^{a}	246^{ab}	1 062^{b}	1 918^{b}
MHA2	40.6^{a}	269^{bcd}	1 189^{cd}	2 071^{bc}
MHA3	40.7^{a}	295^{cde}	1 209de	2 146^{cd}
MHA4	40.7^{a}	309^{e}	1 295^{def}	2 249^{cd}
Pooled SEM	0.35	8.3	24.1	38.6

$^{a, b, c, d, e, f}$ Means within a column not sharing a common superscript were significantly different ($P < 0.05$)

All statistical calculations were performed using Statgraphic Plus package (version 3.1, Statistical graphic Corp., Rockville, MD, USA). The parameters of the exponential model were estimated using Marquardt iterative search method.

RESULTS AND DISCUSSION

Mean body weights of chickens during the experiment are summarized in Tab. III. In general, the weight of broilers gradually increased with increasing levels of both methionine sources, thus demonstrating a clear SAA deficiency of the basal diets. The growth rate of chickens receiving diets supplemented with 0.16 or 0.28% DLM was equal to or better than the values given in the Ross 308 performance objectives (Ross 308 Broiler Performance Objectives, 2012). The response to corresponding MHA-FA supplements was numerically lower in most cases, but the differences as assessed by the Tukey HSD test were not significant suggesting no differences between efficiency of products. However, this comparison can result in misleading conclusion. As Hoehler (2006) pointed out, the comparison of pairs of treatments from a dose-response design cannot explain how much of a nutrient source is needed to replace another one without affecting performance. In addition, ANOVA and multiple range tests are often not sensitive enough to detect small differences such as between treatments DLM3 and DLM4. The small difference between DLM3 and DLM4 levels indicated a diminishing returns pattern of the dose-response relationship and suggested that SAA intake was close to the requirement in these groups. Similar results were obtained with feed conversion ratio (Tab. IV). The data for the whole experiment showed better feed conversion in chickens fed DLM-supplemented diets, but the improvement over the MHA-FA counterparts was insignificant. Moreover, at the highest inclusion level, the performance of broilers were similar using either products.

The changes in carcass and breast meat yields in response to methionine source supplements are shown in Tab. V. Similar response have been observed in other studies (Schutte and Pack, 1995; Wallis, 1999; Lemme *et al.*, 2002; Ahmed and Abbas, 2011). The increase in breast meat yield is assumed to be due at least partly to decreased fat deposition observed in most experiments with methionine supplements (Schutte and Pack, 1995; Wallis, 1999; Ahmed and Abbas, 2011). It seems that these changes might be due to the redistribution of dietary energy towards higher protein deposition resulting from better amino acid balance or the stimulating effect of methionine on the oxidative catabolism of fatty acids via its participation in carnitine synthesis (Schutte *et al.*, 1997). Methionine-induced alterations of metabolic pathways controlling lipogenesis may also be involved (Takahashi and Akiba, 1995). Zhai *et al.* (2012) suggested that the effect of methionine on

IV: *Feed conversion ratios of chickens during experiment*

Treatment	Time interval (days)			
	1–10	11–24	25–35	1–35
Basal	1.467[a]	1.585[a]	2.318[a]	1.908[a]
DLM1	1.287[bc]	1.500[ab]	2.080[ab]	1.730[bc]
DLM2	1.282[bc]	1.401[bc]	1.994[b]	1.641[cd]
DLM3	1.263[bc]	1.392[bc]	1.924[b]	1.612[d]
DLM4	1.201[c]	1.383[c]	1.973[b]	1.601[d]
MHA1	1.347[ab]	1.493[abc]	2.112[ab]	1.749[b]
MHA2	1.308[bc]	1.435[bc]	2.063[ab]	1.687[bcd]
MHA3	1.216[bc]	1.464[bc]	1.955[b]	1.648[bcd]
MHA4	1.199[c]	1.414[bc]	1.934[b]	1.610[d]
Pooled SEM	0.0291	0.0246	0.0638	0.0230

[a, b, c, d] Means within a column not sharing a common superscript were significantly different (P < 0.05)

V: *Results of carcass analysis*

Treatment	Body weight (g)	Carcass yield (% LW)	Breast meat yield (% LW)
Basal	1736.0[a]	64.28[a]	14.88 [a]
DLM1	2067.3[bc]	66.97[bc]	17.62 [bc]
DLM2	2248.3[cd]	68.31[cd]	19.29 [def]
DLM3	2302.7[d]	68.52[cd]	19.62 [def]
DLM4	2343.3[d]	68.62[cd]	19.87 [ef]
MHA1	1996.3[b]	65.97[b]	16.89 [b]
MHA2	2158.3[bcd]	67.32[bc]	18.25 [bcd]
MHA3	2230.7[cd]	68.08[cd]	18.88 [cde]
MHA4	2321.0[d]	68.56[cd]	19.73 [def]
Pooled SEM	44.2	0.36	0.33

[a, b, c, d, e, f] Means within a column not sharing a common superscript were significantly different (P <0.05)

muscle protein deposition might have been due to sarcoplasmic rather than myofibrillar hypertrophy.

Because of the diminishing returns pattern observed in all the response criteria studied, exponential model by Littell *et al.* (1997) was used to estimate the bioavailability of MHA-FA relative to DLM. The comparison was made on product basis. The parameters of the model with their standard errors and confidence intervals for various response criteria are summarized in Tab. VI. As evidenced by the R-squared statistic, the model fitted the experimental data well for both methionine sources, explaining 73–82 % of total variability in the response. As shown in Figs. 1 and 2, the response of chickens to DLM supplements was superior to that achieved with MHA-FA, particularly at low levels of supplementation. It is well known that the response to a limiting amino acid (and therefore the sensitivity of an assay) diminishes as its supply approaches optimum requirement (Fisher *et al.*, 1973; Fuller and Garthwaite, 1993). Fitting experimental data to the exponential model enables to estimate real availability values, representing the whole range of DLM and MHA-FA intake. The RBV of MHA-FA in terms of body weight estimated by this way was 0.68, i.e. 68 % as compared with DLM (100 %) (Fig. 1 A). The same value was calculated when body weight gain was used as a criterion of response (data not given). The upper 95 % confidence interval of the c_2/c_1 ratio was higher than 0.88 (Tab. VII), thus indicating an insignificant difference between DLM and MHA-FA

VI: *Parameters of exponential model* describing the relationship between response criteria and supplementary levels of two methionine sources*

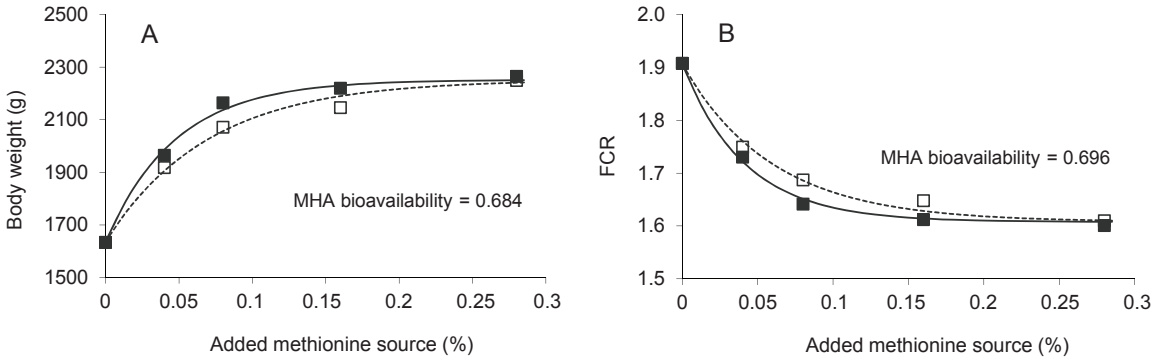
Criterion of response	Parameter	Estimate	Standard error	95% confidence interval		R^2 (%)
				Lower	Upper	
Body weight (g)	a	1.6366	0.0366	1.5632	1.7101	82.04
	b	0.6152	0.0408	0.5332	0.6972	
	c_1	–20.9374	3.6206	–28.2097	–13.6651	
	c_2	–14.3287	2.3733	–19.0967	–9.5617	
Feed conversion ratio	a	1.9050	0.0221	1.8607	1.9494	74.90
	b	–0.2974	0.0245	–0.3465	–0.2483	
	c_1	–23.9639	5.1039	–34.2154	–13.7125	
	c_2	–16.6680	3.3458	–23.3884	–9.9477	
Carcass yield (% of live weight)	a	64.2263	0.3453	63.5328	64.9200	72.92
	b	4.4235	0.3846	3.6510	5.1960	
	c_1	–26.1890	6.1906	–38.6237	–13.7550	
	c_2	–13.6680	2.9107	–19.5143	–7.8218	
Breast meat yield (% of live weight)	a	14.8819	0.3131	14.2530	15.5107	80.05
	b	4.9399	0.3508	4.2353	5.6446	
	c_1	–22.6410	4.2976	–31.2730	–14.0090	
	c_2	–12.8680	2.2789	–17.4450	–8.2905	

*Function being fitted: $y = a + b*(1 - \exp(c_1*DLM + c_2*MHA))$

VII: *Summary of relative bioavailability values (RBV) of MHA -FA according to various criteria calculated on product or equimolar basis and their 95 % confidence intervals (%, DLM = 100))*

Criterion of response	Product basis		Equimolar basis	
	RBV	Confidence interval[1]	RBV	Confidence interval[1]
Body weight	68.4	45.1–91.8	77.7	51.2–104.3
Feed conversion ratio	69.5	39.0–100.1	79.0	44.3–113.7
Carcass yield	52.2[2]	27.5–76.8	59.3[3]	31.3–87.3
Breast meat yield	56.8[2]	35.8–77.9	64.6[3]	40.7–88.4

[1]Calculated after reparametrization of the exponential model
[2]Significantly different from 88 %
[3]Significantly different from 100 %

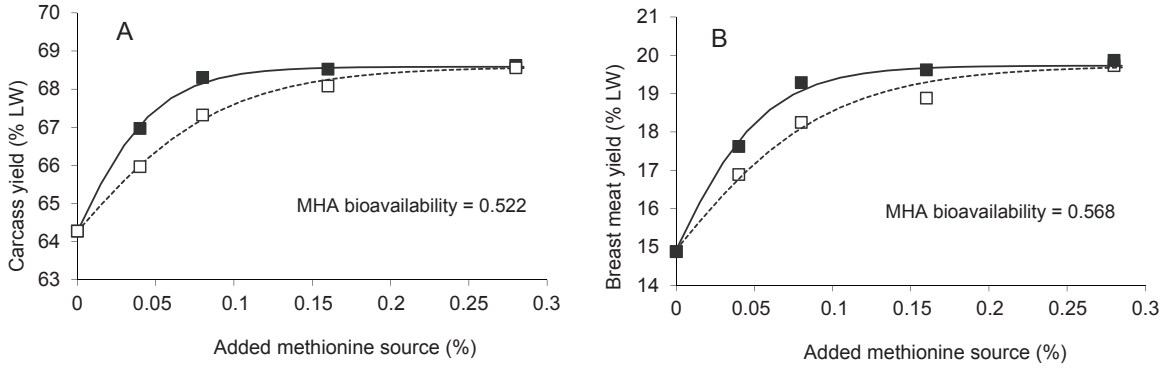


1: *Body weights (A) and feed conversion ratios (B) of chickens at 35 days of age fed increasing amounts of DLM (■) or MHA (□). Plotted from the equations: (A) y = 1.6366 + 0.6152*(1 – exp(–20.937*x_1 – 14.329*x_2)). R^2 = 80.0; (B) y = 1.9050 – 0.2974*(1 – exp(–23.964*x_1 – 16.668*x_2)). R^2 = 74.9*

availability on equimolar basis. Similar values of relative MHA-FA availability were obtained in other studies. Based on 10 experiments reported in the literature, Lemme *et al.* (2002) calculated the average MHA-FA bioavailability value of 69%. A meta-analysis of 46 dose-response experiments by Sauer *et al.* (2008) showed that the RBV of MHA-FA was 81% on equimolar basis, i.e. 71% on product basis. The RBV for feed conversion found in the present study (70%, Fig. 1 B) was slightly higher than that for body weight. In contrast, the mean values calculated by both Lemme *et al.* (2002) and Sauer *et al.* (2008) were lower (66 and 64%, respectively). However, the literature data were rather variable in this respect, ranging from 51% (Lemme *et al.*, 2002; Payne *et al.*, 2006) up to 73% (Esteve-Garcia and Llaurado, 1997) and 76% (Jansman *et al.*, 2003). The RBV was not significantly different from 0.88 in the present study (Tab. VII).

When carcass yield was used as an independent variable in the exponential model, the resulting RBV was estimated to be 0.52 (Fig. 2 A). The 95% confidence interval for c_2/c_1 ratio (27.5–76.8, Tab. VII) demonstrated that the biological availability of the active substance of MHA-FA (88% by weight) was significantly lower than that of DLM. Breast meat yield response to DLM or MHA-FA additions followed a similar pattern (Fig. 2 B). The estimated MHA-FA availability was 56.8% (35.8–77.9) and was significantly less than 88%. Despite the differences in body weight and methods of carcass evaluation, the present data on breast meat yield agree relatively well with the results of other studies. On product basis, the RBVs of MHA-FA were reported to be 45% (Esteve-Garcia and Llaurado, 1997), 48–54% (Payne *et al.*, 2006), 53–64% (Lemme *et al.*, 2002) and 63% (Wallis, 1999). In most cases, the bioavailability of MHA-FA was significantly lower than that of DLM.

Considering the concentration of active substance in DLM (99%) and liquid MHA-FA (88%) and the molecular weight (DLM 149.21 g/mol; MHA-FA 150.20 g/mol), the relative bioavailability values as well as their confidence intervals estimated on product basis can be recalculated to equimolar basis multiplying the respective values by (0.99 * 150.20)/(0.88 * 149.21). The data given in Tab. VII show that, in comparison with equimolar levels of DLM, the MHA-FA availability was lower in all cases, the greatest difference being found in carcass and breast meat yields. The sensitivity of both absolute and relative breast meat yield to dietary methionine levels has been demonstrated in many studies (Schutte and Pack, 1995; Zhai *et al.*, 2012). Also, it has been shown that the optimal levels of SAA (as well as lysine) for breast meat deposition are higher than for body weight gain (Bartov and Plavnik, 1998). The clear-cut response of breast meat yield to DLM supplements is of particular importance, since the proportion of breast meat in portioned birds may have a great impact on overall profitability in poultry industry (Pack *et al.*, 2003).



2: *Carcass yields (A) and breast meat yields (B) of chickens at 35 days of age fed increasing amounts of DLM (■) or MHA (□). Plotted from the equations: (A) y = 64.226 + 4.424*(1 – exp(–26.189*x_1 – 13.668*x_2)). R^2 = 72.9; (B) y = 14.882 + 4.940*(1 – exp(–22.641*x_1 – 12.868*x_2)). R^2 = 80.1*

Various hypotheses have been proposed to explain lower bioavailability of MHA-FA relative to DLM. The commercial MHA-FA product contains about 65% monomers and 23% of dimers and oligomers. It has been suggested that non-monomeric forms of MHA-FA have a lower bioavailability than monomers (Van Weerden *et al.*, 1992). In contrast, Martín-Venegas *et al.* (2006) found that the presence of oligomers is not the limiting factor in MHA-FA utilization. The difference in the bioavailability of both methionine sources may also be explained by a decreased intestinal absorption of MHA-FA relative to DLM as suggested by Lingens and Molnar (1996) and Maenz and Engele-Schaan (1996). This might be due to the different absorption mechanisms of both methionine sources (Maenz and Engele-Schaan, 1996) or the interaction of MHA-FA with gut microflora (Drew *et al.* 2003). The latter authors, using ^{3}H-labelled L-forms of methionine and MHA-FA, demonstrated that intestinal bacteria significantly reduced the apparent absorption of MHA-FA from the intestinal tract of broiler chickens. Another potential hypothesis on the inferior bioavailability of MHA-FA was proposed by Dilger and Baker (2008) and Baker (2009), who found that, at severe deficiency of methionine, the utilization of both MHA-FA and its calcium salt was lower in the presence than absence of excess cysteine. The authors concluded that cysteine to methionine ratio might be an important factor affecting the bioavailability of MHA-FA relative do DLM.

The exponential model used in the present study was based on an assumption of a common asymptote for both methionine sources. This assumption has been questioned by Kratzer and Littell (2006) who suggested to use two separate models (with different asymptotes) when comparing DLM and MHA availability. In contrast, Piepho (2006) considered the conclusions by Kratzer and Littell (2006) not fully justified from a statistical point of view. To confront these two approaches, separate exponential models were fitted to carcass and breast meat yield data (recalculated to equimolar basis) obtained in the present study as suggested by Kratzer and Littell (2006). The resulting RBV for carcass yield was almost identical with that obtained with the common asymptote model (59.7 vs. 59.3%) while the RBV for breast meat yield was about 8% higher (69.6 vs. 64.6 %). In both cases, however, the values were considerably lower than 100%, thus indicating lower bioavailability of MHA-FA.

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