# Nutritional value of methionine hydroxy analogue calcium salt compared with both pure DL-methionine and diluted DL-methionine with 65% purity

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**Correct use of DL-methionine (DL-Met) and methionine hydroxy analogue calcium salt (MHA-Ca) requires knowledge on their nutritional value which can be determined by multi-exponential regression analysis of dose response data. In order to both examine the biological value of MHA-Ca compared with DL-Met and to validate the mathematical approach a dose-response trial was performed with graded levels of either DL-Met, MHA-Ca or DL-Met-65 the latter being DL-Met diluted to a purity of 65%. It was hypothesised that the biological effectiveness of DL-Met-65 must be about 65% if the regression method is valid. Basal unsupplemented starter, grower and finisher diets contained 0.63, 0.57, and 0.52% Met+Cys, respectively, and were fed to male Ross 308 chicken. Regarding weight gain, feed conversion ration (FCR) and breast meat yield (BMY), broilers responded significantly and non-linearly to either Met source. Biological effectiveness of MHA-Ca compared with DL-Met was on average 60% (gain-65%; FCR-60%; BMY-53%) whilst that of DL-Met-65 was 62% (61%; 62%; 63%). These findings suggest that 1kg MHA-Ca can be replaced by 650g or less DL-Met for achieving the same animal performance. The determined bio-efficiency of DL-Met-65 was close to 65% supporting the mathematical approach to be suitable for bio-efficiency determination.**

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Keywords: DL-methionine; Methionine hydroxy analogue calcium salt; bio-efficiency; broiler

## Introduction

Supplementing methionine sources to broiler diets to balance the dietary amino acid profile in accordance to the broiler's requirement or the economic optimum is common practice. For this purpose two products are available traditionally: DL-Methionine (DL-Met) and the liquid methionine hydroxy analogue free acid (MHA-FA). Recently, a third product was re-introduced to the market. Methionine hydroxy analogue calcium salt (MHA-Ca) is a powder containing at least 84 % monomeric hydroxy analogue, 14 % calcium and 2 % water. Similar to the debate about the relative efficacy of liquid MHA-FA the nutritionist needs to know*evaluate* the replacement ratio between MHA-Ca and DL-Met to properly formulate diets.

Therefore, a simultaneous dose-response broiler trial was performed allowing for the determination of the efficacy of MHA-Ca relative to DL-Met by regression analysis. In addition to graded levels of DL-Met and MHA-Ca also graded levels of diluted DL-Met (DL-Met-65) were tested. For this, DL-Met was diluted with corn starch to a purity of 65 %. It was hypothesised that the relative efficacy of this product must be around 65 %. Therefore, DL-Met-65 served as an internal standard to validate the methodology of multi-exponential regression analysis.

## Materials and methods

A total of 1920 male day old Ross 308 broiler chicken with an average body weight of 41.6 g were equally distributed to 96 floor pens with 20 chicks each. Pens were initially covered with wood shavings, later on straw was used for bedding. Illumination and temperature regimes followed recommendations (Aviagen, 2002) and German animal welfare regulations. Each pen (3 $m^2$) was equipped with an automatic feeder and round drinker for *ad libitum* supply of feed and water. The feeding program included a starter (1-10 d), a grower (11-27 d) and a finisher phase (28-38).

**Table 1 Ingredients and nutrient composition of the basal starter, grower, and finisher diet.**

| Ingredients, g/kg | Starter | Grower | Finisher | Energy and nutrients, % | Starter | Grower | Finisher |
|---|---|---|---|---|---|---|---|
| Wheat | 202.2 | 465.6 | 594.7 | Energy, MJ ME/kg | 12.6 | 13.3 | 13.4 |
| Corn | 200.0 | - | - | | | | |
| Soybean meal (48) | 389.7 | 291.6 | 227.6 | Crude Protein | 24.3 | 21.5 | 19.6 |
| Peas | 100.0 | 124.1 | 159.6 | Lysine | 1.43 | 122 | 1.11 |
| Soybean oil | 59.7 | 79.4 | 77.8 | **Met + Cys** | **0.74** | **0.66** | **0.60** |
| Biolys®* | 1.09 | 1.14 | 1.34 | Threonine | 0.95 | 0.82 | 0.76 |
| L-Threonine | 0.40 | 0.44 | 0.62 | Arginine | 1.68 | 1.46 | 1.33 |
| Calcium carbonate | 14.3 | 12.5 | 12.7 | | | | |
| Mono-Ca-phosphate | 22.0 | 16.0 | 16.4 | SID Lysine** | 1.28 | 1.09 | 0.99 |
| Salt (NaCl) | 2.65 | 2.70 | 2.70 | **SID Met + Cys** | **0.63** | **0.57** | **0.52** |
| Na-bicarbonate | 2.93 | 1.51 | 1.52 | SID Threonine | 0.81 | 0.70 | 0.65 |
| Vitamin-Mineral-Enzyme Premix | 5.00 | 5.00 | 5.00 | SID Arginine | 1.55 | 1.33 | 1.20 |

* Biolys®: 50.7% L-lysine; **SID: Standardised ileal digestible

The experimental arrangement comprised 16 dietary treatments each of which was replicated 6 times. In treatment 1 basal, Met+Cys deficient starter, grower and finisher diets were fed to the birds (Table 1). In treatments 2 to 6 DL-Met was gradually supplemented to the basal diets (0.03, 0.06, 0.10, 0.15, 0.21 % of diet). Experimental diets 7 to 11 contained increasing levels of MHA-Ca (0.03, 0.06, 0.10, 0.15, 0.21 % of diet corresponding to equimolar levels of 0.025, 0.050, 0.084, 0.126, 0.176 % assuming a content of 84 % in the product MHA-Ca). Experimental diets 12 to 16 were supplemented with increasing levels of diluted DL-Met (DL-Met-65; 0.03, 0.06, 0.10, 0.15, 0.21 % of diet corresponding to 0.020, 0.039, 0.065, 0.098, 0.137 % pure DL-Met). In order to derive DL-Met-65 pure DL-Met was diluted with corn starch. Analysis of the experimental starter, grower and finisher diets confirmed the expected concentrations. Feeds were offered as crumbles (starter) and pellets (2.5, 3.0 mm)

Body weights and feed consumption were recorded after each phase and subsequently daily weight gain and feed conversion were computed. After termination of the experiment 4 birds per pen with body weights close to the pen average were selected for breast meat (including bone and skin) yield determination which was expressed in percent of carcass weight.

Data were analysed by analysis of variance and the Tukey-Kramer test was applied to account for multiple comparisons between treatments. In order to determine the effectiveness of both MHA-Ca and DL-Met-65 relative to DL-Met data were analysed by multi-regression analysis as proposed by Littell et al (1997). The level of significance was chosen at $p<0.05$.

## Results and discussion

Broilers performed well and losses were low (3.2 %). At highest DL-Met, MHA-Ca, and DL-Met-65 supplementation average final body weights were 2813, 2788, and 2781 and thus about 20 % higher than expected (Aviagen, 2002). Final weight achieved with the basal diet was only 1725 g but performance improved with increasing supplementation of either product. This is reflected in average daily gain as presented in Table 2. For all products daily gain improved significantly already with the first inclusion level demonstrating the degree of Met+Cys deficiency in the basal diet. However, regarding the 0.03 and 0.06 inclusion levels daily gain of the DL-Met treatments was significantly higher than the corresponding MHA-Ca and DL-Met-65 treatments suggesting a high efficacy of DL-Met. Although also feed intake, feed conversion ratio, and breast meat yield improved significantly with increasing supplementation there were no significant differences between the corresponding supplementation levels indicating that ANOVA is not a proper method to detect differences in relative effectiveness of methionine sources.

**Table 2 Weight gain, feed intake, feed conversion ratio, and breast meat yield of 1 to 38 days old male Ross 308 broilers fed graded levels of DL-Met, MHA-Ca and DL-Met-65 supplemented to a control diet on**

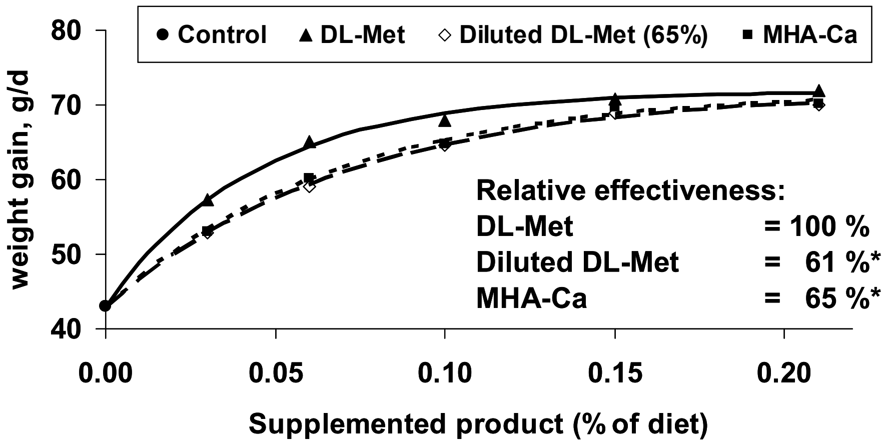
| | Treatment | inclusion | Weight gain g/d | Feed intake g | Feed per gain kg/kg | Breast meat % of carcass |
|---|---|---|---|---|---|---|
| 1 | Control | 0.00 | 44.32 [a] | 97.6 [a] | 2.202 [f] | 29.07 [a] |
| 2 | DL-Met - | 0.03 | 59.02 [c] | 112.4 [bcd] | 1.904 [cde] | 32.07 [bc] |
| 3 | DL-Met - | 0.06 | 67.13 [d] | 120.4 [cde] | 1.795 [abcd] | 33.02 [bcde] |
| 4 | DL-Met - | 0.10 | 70.02 [de] | 120.9 [cde] | 1.729 [abc] | 34.82 [efg] |
| 5 | DL-Met - | 0.15 | 72.93 [ef] | 125.2 [e] | 1.717 [abc] | 35.25 [fg] |
| 6 | DL-Met - | 0.21 | 74.02 [f] | 123.6 [de] | 1.671 [a] | 35.96 [g] |
| 7 | MHA-Ca - | 0.03 | 54.60 [b] | 110.2 [bc] | 2.021 [ef] | 31.29 [b] |
| 8 | MHA-Ca - | 0.06 | 61.98 [c] | 113.5 [bcde] | 1.832 [abcde] | 32.86 [bcde] |
| 9 | MHA-Ca - | 0.10 | 66.80 [d] | 118.5 [bcde] | 1.775 [abc] | 32.97 [bcde] |
| 10 | MHA-Ca - | 0.15 | 71.68 [ef] | 121.3 [cde] | 1.691 [ab] | 33.78 [cdef] |
| 11 | MHA-Ca - | 0.21 | 72.25 [ef] | 123.3 [de] | 1.706 [ab] | 34.40 [defg] |
| 12 | DL-Met 65% - | 0.03 | 54.48 [b] | 107.5 [ab] | 1.975 [de] | 31.50 [b] |
| 13 | DL-Met 65% - | 0.06 | 60.75 [c] | 114.5 [bcde] | 1.885 [bcde] | 32.62 [bcd] |
| 14 | DL-Met 65% - | 0.10 | 66.48 [d] | 121.9 [cde] | 1.833 [abcde] | 33.10 [bcde] |
| 15 | DL-Met 65% - | 0.15 | 70.93 [ef] | 119.1 [bcde] | 1.681 [a] | 35.10 [fg] |
| 16 | DL-Met 65% - | 0.21 | 72.12 [ef] | 122.0 [cde] | 1.692 [ab] | 34.43 [defg] |
| | pooled s.e.m. | | 0.749 | 2.44 | 0.0388 | 0.398 |

Plotting the data against product inclusion levels demonstrated that the dose responses of all three products on weight gain, feed conversion ratio and breast meat yield were of non-linear nature following the law of diminishing returns (Figure 1, breast meat yield not shown). Therefore, multi-exponential regression analysis was applied to determine the efficacy of MHA-Ca and DL-Met-65 relative to DL-Met. Accordingly, MHA-Ca was 65, 60 and 53 % as efficient as DL-Met in order to achieve the same weight gain, feed conversion, and breast meat yield, respectively. All estimates were significantly lower than 84 % which would be the theoretical maximum because of the purity of 84 % of MHA-Ca. An efficiency of MHA-Ca for feed conversion lower than that for weight gain has been reported earlier by Lemme (2004) who analysed data of 33 dose-response experiments in a literature survey. From these 33 trials the relative efficacy of MHA-Ca as compared to DL-Met for weight gain and feed conversion ratio averaged 65.4 and 62.2 % (Lemme, 2004).

Diluted DL-Met-65 was 61, 62, and 63 % as efficient as DL-Met regarding weight gain, feed conversion ratio, and breast meat yield. Considering a certain biological variation it is concluded that these values were close to 65 % which was the expected value because the product was diluted to this percentage. It is, therefore, further concluded that the current results confirm the multi-exponential regression analysis to be suitable. Similar validations of this kind have been reported elsewhere (Lemme et al., 2002; Hoehler et al., 2005).

Based on the analysis of the performance data the results of the current trial can be summarized as follows:

1. The relative efficacy of MHA-Ca was at maximum 65 % compared to DL-Met
2. The relative efficacy of MHA-Ca determined for feed conversion and breast meat yield was lower than that for weight gain
3. Multi-exponential regression analysis is a suitable method to determine the effectiveness of MHA-Ca relative to DL-Met.



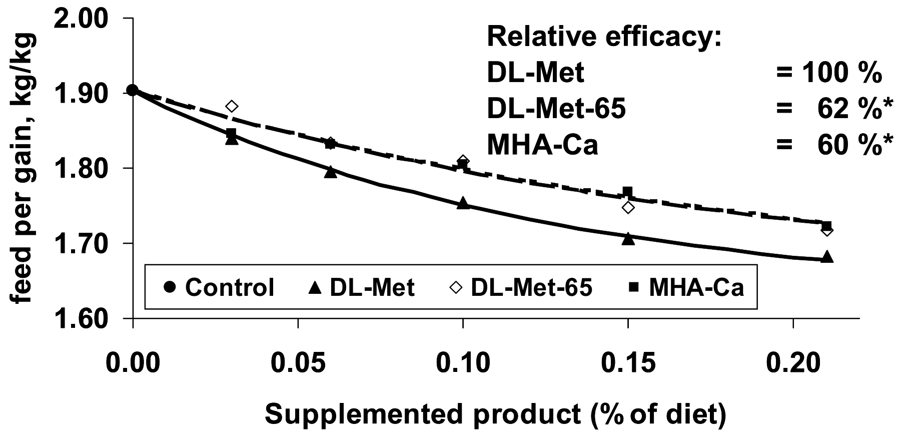




**Figure 1 Responses of male 1 – 38 day old broilers on daily weight gain (top) and feed conversion ratio (bottom) to increasing dietary levels of DL-Met, MHA-Ca, and diluted DL-Met-65. Relative efficacy was determined by multi-exponential regression analysis: $Y = 42.9 + 28.9 * (1 - EXP(-22.77\ DL\text{-}Met - 14.81\ MHA\text{-}Ca - 13.87\ DL\text{-}Met\text{-}65))$ for weight gain and $Y = 1.903 – 0.279 * (1 – EXP(-7.84\ DL\text{-}Met – 4.72\ MHA\text{-}Ca – 4.82\ DL\text{-}Met\text{-}65))$ for feed conversion ratio. All efficacy figures for MHA-Ca and DL-Met-65 were significantly lower than 84 %.**

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