Effectiveness of liquid DL-methionine hydroxy analogue-free acid (DL-MHA-FA) compared to DL-methionine on performance of laying hens

Wirksamkeit von flüssiger DL-Methionin Hydroxyanaloge-Freien Säure (DL-MHA-FA) gegenüber DL-Methionin auf die Leistung von Legehennen

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Introduction

It is well established that the sulphur-containing amino acids (TSAA) and particularly methionine are the first limiting amino acids in commercial laying hen diets. Thus dietary supplementation with free methionine is a common industrial practice and two products, DL-methionine (DL-Met) and liquid DL-methionine hydroxy analogue-free acid (DL-MHA-FA), are commercially available. DL-Met is a dry product containing more than 99% DL-methionine whereas DL-MHA-FA is a liquid containing 88% of a mixture of mono-, di- and oligomers of hydroxy analogue molecules. From a chemical point of view DL-MHA-FA does not represent a complete amino acid and has to be transformed to methionine by transamination.

There is an ongoing discussion on the biological effectiveness of DL-MHA-FA compared to DL-Met. According to Littell et al. (1997) the relative effectiveness of a test substance is the ratio of the dietary amount of a reference substance to the dietary amount of the test substance producing the same performance. This ratio must be invariant over a range of inclusion levels for both products. When the dose-response pattern follows the law of diminishing returns, which basically is the case for amino acid supply, exponential regression analysis is the proper way to compare responses. Taken this into consideration the relative effectiveness of DL-MHA-FA compared to DL-Met on a product-to-product basis has been reported to be around 65% in broilers, clearly different from the content of 88% of active substance in liquid DL-MHA-FA (Pack, 1994). With respect to laying hens, information on this topic is scarce. However, a previous study with laying hens was performed in which DL-Met and liquid DL-MHA-FA were supplemented according to the ratio of 65 units (0.17% DL-Met) to 100 units (0.26% liquid DL-MHA-FA) (Klein and Bertram, 1992). Since there was no difference in performance between both products, the hypothesis of a relative effectiveness of 65% for liquid DL-MHA-FA compared to DL-Met was confirmed. The present study also set out with the working hypothesis that liquid DL-MHA-FA is 65% as effective as DL-Met. However, the products were compared at different supplementation levels. Moreover, the dose-response design allowed verification of the relative effectiveness of liquid DL-MHA-FA compared to DL-Met by exponential regression. In addition, the general effect of the methionine or TSAA level on plumage condition and cannibalism was investigated.

Materials and Methods

A 24-week experiment was performed with a total of 624 laying hens of the ‘Lohmann Selected Leghorn’ strain (LSL). The hens were housed in wire cages with 3 birds per cage. The lighting programme, the temperature and the relative humidity were the same as those practised commercially in Europe. Because hens in two cages had access to the same feeder, one experimental unit consisted of 6 hens. The experiment, therefore, consisted of 8 treatments of 13 replicates each (Table 1).

During the rearing period until week 19, the birds received a commercial pullet diet containing 15% crude protein. From weeks 19 to 21, the hens were adapted to layer feed containing 17% crude protein followed by the experimental period lasting 24 weeks. Hens of treatments I and V (Table 1) were fed the basal diet being deficient in TSAA but matching or exceeding minimum requirements of all other nutrients and energy (GfE, 1999). The methionine and TSAA contents of the basal diet were calculated to be 0.23% and 0.51%, respectively. Three graded levels of either DL-Met¹ (II-IV) or DL-MHA-FA² (VI-VIII) were ad-

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1 DL-methionine, Degussa AG, Germany
2 Alimet®, Novus Europe, Belgium
ded in such a way that the complementary inclusion level of DL-Met (0.05, 0.10, 0.15%) corresponded to 65% of that of liquid DL-MHA-FA (0.077, 0.154, 0.231%). The basal diet (Table 2) was formulated according to the analysed amino acid compositions of the batches of corn, barley and soybean meal. The amount of feed required for each of the 8 treatments was prepared in two batches before commencing the study. Diets were fed as mash. Feed and water were supplied ad libitum.

Crude nutrients, calcium and phosphorus were determined according to the methods described by Naumann et al. (1997). Amino acids in the ingredients, the experimental diets as well as the supplemented DL-Met were analysed in accordance to the Commission Directive 98/64/E.C. (1998). Supplemented liquid DL-MHA-FA was determined following the method of Naumann et al. (1997). The analysed contents of nutrients, total and supplemented amino acids in the experimental diets were in good agreement with calculated data.

Throughout the experiment egg production was recorded daily whereas mean egg weight was determined at four-week intervals. Feed consumption was determined for the period 22–25, 26–29, 30–34 and 35–45 weeks of age. Based on these data daily egg mass (g/hen/d) and feed conversion ratio (kg feed/kg egg mass) were calculated for the entire experiment. Body weight of the hens was recorded at 19 and 45 weeks of age to calculate body weight gain (g). The feathering of the hens was scored at the end of the experiment using scores from 0 (no damage) to 5 (totally denuded) (Bessel, 1984). Five body areas (head and neck, back, tail, wings, belly) were scored separately and finally averaged. All cages were checked daily for mortality and mortality caused by cannibalism was recorded separately.

Statistical analysis of the experimental data was performed by an ANOVA procedure of JMP (1995). Differences between the means were tested for significance by the Student-Newman-Keuls-Test. Regarding daily egg mass and feed conversion ratio, the relative effectiveness of liquid DL-MHA-FA compared to DL-Met was determined by an exponential regression analysis (Littell et al., 1997).

### Results and discussion

The laying hens responded significantly to the supplementation (Table 3) demonstrating that the products were tested in a sensitive range which is an important prerequisite for determining the biological effectiveness. The performance level was high with a mean egg production, egg weight and daily egg mass of 95.1%, 61.4 g, and 58.4 g/hen, respectively, at the highest inclusion level. Compared to the basal diet average production increased by 8% at the highest inclusion level of either DL-Met or DL-MHA-FA. Accordingly, egg weight improved by 6% resulting in an increase of 14% in daily egg mass. These effects were mirrored by the feed conversion ratio because feed intake – ranging between 109 and 113 g/hen/d – was not significantly affected by supplemental DL-Met or DL-MHA-FA. Interestingly, there was still a response to supplementation...

### Table 2. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>35.65</td>
</tr>
<tr>
<td>Barley</td>
<td>30.55</td>
</tr>
<tr>
<td>Soybean meal (48% Crude Protein)</td>
<td>18.94</td>
</tr>
<tr>
<td>Limestone</td>
<td>9.09</td>
</tr>
<tr>
<td>Dicalciumphosphate</td>
<td>1.17</td>
</tr>
<tr>
<td>Na-Bicarbonate</td>
<td>0.13</td>
</tr>
<tr>
<td>Ca-Propionate</td>
<td>0.40</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.27</td>
</tr>
<tr>
<td>Soybeanoil</td>
<td>0.51</td>
</tr>
<tr>
<td>Palm fat</td>
<td>2.59</td>
</tr>
<tr>
<td>Premix¹</td>
<td>0.71</td>
</tr>
</tbody>
</table>

¹ Supplied per kg diet: vit. A, 14823 IU; vit. D₃, 3706 IU; vit. E, 43 mg; vit. K₃, 3 mg; vit. B₁₂, 4 mg; vit. B₆, 7 mg; vit. B₇, 7 mg; vit. B₁₃, 37 mcg; nicotinic acid, 62 mg; pantothenic acid, 16 mg; folic acid, 1235 mcg; biotin, 124 mcg; choline chloride, 802 mg; Fe, 90 mg; Mn, 121 mg; Zn, 80 mg; Cu, 15 mg; I, 1.61 mg; Se, 3 mg; vit. B₁, 4 mg; vit. B₂, 7 mg; vit. B₆, 15 mg; l, 1.61 mg; Se, 0.50 mg; Co, 0.60 mg

### Table 3. Effects of DL-Met and liquid DL-MHA-FA on laying hen performance from 22–45 weeks of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Methionine source</th>
<th>Dosage level</th>
<th>Production</th>
<th>Egg weight</th>
<th>Egg mass</th>
<th>Feed intake</th>
<th>Feed conversion</th>
<th>Body weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>DL-Met</td>
<td>1</td>
<td>87.9 ± 3.8</td>
<td>57.5 ± 1.5</td>
<td>50.8 ± 2.8</td>
<td>109 ± 6</td>
<td>2.22 ± 0.09</td>
<td>439 ± 104</td>
</tr>
<tr>
<td>II</td>
<td>DL-Met</td>
<td>2</td>
<td>94.3 ± 3.8</td>
<td>60.4 ± 0.9</td>
<td>57.0 ± 2.0</td>
<td>113 ± 3</td>
<td>2.01 ± 0.07</td>
<td>554 ± 79</td>
</tr>
<tr>
<td>III</td>
<td>DL-Met</td>
<td>3</td>
<td>93.1 ± 4.2</td>
<td>60.9 ± 1.0</td>
<td>56.8 ± 2.8</td>
<td>110 ± 3</td>
<td>1.97 ± 0.10</td>
<td>601 ± 90</td>
</tr>
<tr>
<td>IV</td>
<td>DL-Met</td>
<td>4</td>
<td>94.9 ± 3.6</td>
<td>61.4 ± 1.3</td>
<td>58.3 ± 2.4</td>
<td>109 ± 3</td>
<td>1.89 ± 0.06</td>
<td>538 ± 95</td>
</tr>
<tr>
<td>V</td>
<td>DL-MHA-FA</td>
<td>1</td>
<td>87.8 ± 3.3</td>
<td>58.3 ± 1.6</td>
<td>51.3 ± 2.5</td>
<td>109 ± 7</td>
<td>2.17 ± 0.10</td>
<td>444 ± 102</td>
</tr>
<tr>
<td>VI</td>
<td>DL-MHA-FA</td>
<td>2</td>
<td>93.0 ± 2.2</td>
<td>60.4 ± 1.6</td>
<td>56.1 ± 2.0</td>
<td>112 ± 3</td>
<td>2.03 ± 0.05</td>
<td>518 ± 91</td>
</tr>
<tr>
<td>VII</td>
<td>DL-MHA-FA</td>
<td>3</td>
<td>94.9 ± 2.2</td>
<td>61.4 ± 1.2</td>
<td>58.2 ± 2.1</td>
<td>110 ± 3</td>
<td>1.92 ± 0.04</td>
<td>561 ± 80</td>
</tr>
<tr>
<td>VIII</td>
<td>DL-MHA-FA</td>
<td>4</td>
<td>95.2 ± 1.1</td>
<td>61.3 ± 1.0</td>
<td>58.4 ± 1.5</td>
<td>109 ± 3</td>
<td>1.89 ± 0.05</td>
<td>561 ± 76</td>
</tr>
</tbody>
</table>

### Analysis of variance

<table>
<thead>
<tr>
<th>Source of methionine</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
<th>n.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of methionine</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Source x dose</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

¹ Mean-values ± SD

² n.s. = non significant (P > .05); * P < .05; ** P < .01; *** P < .001

Archiv für Geflügelkunde 3/2002
of both methionine sources regarding feed conversion ratio when the dietary TSAA level exceeded official recommendations (GfE, 1999). This observation is similar to findings of previous studies where the TSAA requirement was found to be higher for maximum efficiency of feed utilisation than for obtaining maximum egg production (Schutte et al., 1994; Bertram et al. 1995). In these latter studies the optimum dietary TSAA content for white and brown layers was estimated to be 0.67% and 0.66% at feed intake levels around 125 g and 110 g, respectively.

With respect to the analysis of variance the factor ‘dose of methionine supplement’ was highly significant for all traits (Table 3). As expected, no differences, neither directly nor as interaction, have been observed between the methionine sources. This is in line with the findings of Klein and Bertram (1992) and confirms the assumption of a relative effectiveness of 65% for liquid DL-MHA-FA since the corresponding treatments (II vs. VI, III vs. VII, IV vs. VIII) were adjusted to this effectiveness figure.

Using exponential regression analysis (Littell et al., 1997) the relative effectiveness of liquid DL-MHA-FA compared to DL-Met on a product-to-product basis was 67% for daily egg mass production (Figure 1) and 69% for feed conversion ratio (Figure 2). Considering the generally higher biological variance of egg production traits as compared to broiler performance traits, especially over a

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**Fig. 1.** Daily egg mass production of laying hens between 22 and 45 weeks of age as affected by source and amount of added methionine

**Fig. 2.** Feed conversion ratio of laying hens between 22 and 45 weeks of age as affected by source and amount of added methionine
long period of 24 weeks, these figures are close to the average relative effectiveness of 65% for DL-MHA-FA compared to DL-Met previously reported for broilers (Pack, 1994). In addition, these figures are in good accordance with responses observed in previous layers studies. Recalculation – using exponential regression analysis – of dose-response data obtained from Reid et al. (1982) and van Weerden et al. (1984) resulted in biological effectiveness figures for liquid DL-MHA-FA of 52% and 61% for daily egg mass and 69% and 55% for feed conversion ratio, respectively. However, the recalculated values determined for both studies by Harms and Russell (1994) were somewhat higher and three of four values ranged between 75% and 83%. Only the relative effectiveness for FCR in experiment 1 of the mentioned paper was unusually high (232%) and was therefore excluded from further calculations. Thus, taking these layer studies together including the present data, liquid DL-MHA-FA was 68% as effective as DL-Met.

Questions remain about the physiological reasons for the results. Several studies with broilers using radiolabelled methionine sources indicated a significantly lower absorption of the hydroxy analogue compared to methionine (Lingens and Molnar, 1996; Maenz and Engele-Schaan, 1996). This might be due to degradation of a substantial fraction of the hydroxy analogue by microbial fermentation during passage through the small intestine (Lemme et al., 2001). Moreover, earlier studies performed by Saunders (1991) provided strong evidence that especially the oligomers of liquid DL-MHA-FA are poorly absorbed and van Weerden et al. (1992) showed a significantly lower relative effectiveness of DL-MHA-oligomers compared to the commercial product as a mix of mono-, di-, and oligomers, which in turn was 66% as efficacious as DL-Met. Furthermore, both products are absorbed by specific active absorption mechanisms while absorption by simple diffusion can be neglected (Maenz and Engele-Schaan, 1996). There is evidence that the mechanism responsible for the uptake of DL-MHA is less efficient (Maenz and Engele-Schaan, 1996). Finally, it should be kept in mind that the hydroxy analogue molecules have to be converted to methionine before intermediate use and incorporation (Saunderson, 1991).

Apart from the performance criteria, body weight gain was positively affected by supplementation of either DL-Met or liquid DL-MHA-FA (Table 3). Birds fed the highest inclusion level gained about 100 g more than those fed the basal diet suggesting again a clear methionine or TSAA deficiency of the basal diet. Mortality rates led to the same conclusion since a mortality of 16% recorded for the basal treatment could clearly be reduced by methionine supplementation (Table 4). Interestingly, as much as 75% of the mortality in treatments I and V was caused by cannibalism. Together with the tendency that plumage condition score improved with increasing DL-Met or liquid DL-MHA-FA levels (Table 4), this strongly indicates that a methionine deficiency may result in a poor plumage condition and high mortality due to cannibalism. This is in line with results of Amirosen and Petersen (1997) who reported a deterioration of plumage condition in layers and high mortality especially caused by cannibalism with decreasing dietary crude protein content. They suggested that both deficiency of essential amino acids and/or an amino acid imbalance might have caused these effects in the low protein treatments.

### Summary

A two-factorial experiment was conducted to compare the effect of supplemented DL-methionine (DL-Met) or liquid DL-methionine hydroxy analogue-free acid (DL-MHA-FA) on performance in laying hens. The calculated methionine- and Met + Cys-content of the basal diet were 0.23% and 0.51%, respectively. Three graded levels of DL-Met were supplemented in a way that the complementary inclusion level of DL-Met (0.05, 0.10, 0.15%) corresponded to 65% of that of liquid DL-MHA-FA. The experimental period was 24 weeks, from 22–45 weeks of age. The relative effectiveness of liquid DL-MHA-FA compared to DL-Met was found to be 67% and 69% respectively with regard to daily egg mass and feed conversion ratio. In addition results provide evidence that methionine deficiency deteriorates plumage condition and increases mortality particularly by cannibalism.

### Keywords

Laying hen, nutrition, methionine, hydroxy analogue, DL-methionine, effectiveness
Zusammenfassung

Wirksamkeit von flüssiger DL-Methionin Hydroxyanalogue-Freeien Säure (DL-MHA-FA) gegenüber DL-Methionin auf die Leistung von Legehennen


Stichworte

Legehennen, Ernährung, Methionin, Hydroxyanalog, DL-Methionin, Wirksamkeit

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