Effect of different dietary levels of DL-methionine and the calcium salt of DL-2-hydroxy-4-[methyl] butanoic acid on the growth performance, carcass yield and meat quality of broiler chickens


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Introduction

Methionine is the first limiting amino acid in common broiler chicken and turkey diets. Dietary supplementation with methionine increases protein utilisation and consequently feed utilisation and improves performance. A study of fast-growing turkeys, conducted by Kubińska et al. (2014), demonstrated that immune system function can be modulated by increasing dietary methionine concentrations. However, if the dietary supply of methionine is low, the body synthesises adequate quantities of the semi-essential amino acid cysteine from methionine and both amino acids participate in protein synthesis (Gardzielewska et al., 2005). According to many authors, methionine as well as methionine + cysteine levels in poultry diets are too low (Wallis, 1999; Café and Waldroup, 2006). Recent studies have shown that a dietary methionine deficiency significantly inhibits broiler growth, compared to a deficiency of other essential amino acids (Carew et al., 1997; Elwert et al., 2008), whereas excess methionine intake decreases feed intake, thus reducing the body weight gains of birds (Hesabi et al., 2006; Cengiz et al., 2008). The above indicates that adequate dietary methionine levels are required to increase lean carcass content and to reduce abdominal fat deposition in broilers (Liu et al., 2010). In all poultry production systems, feed cost is the largest single cost item accounting for up to 70–75% of total production costs per bird. Thus, it is important to find cost-effective feed ingredients for poultry. The optimum level and source of methionine have important implications for the growth performance of broilers. Economically optimal methionine levels in poultry diets should be determined in view of the maximum performance of birds, current feed prices and the income from meat sales. Over the last few decades, DL-methionine (DLM) and DL-2-hydroxy-4-[methyl] butanoic acid (DL-HMB), known as liquid methionine hydroxy-analogue free acid (MHA-FA), have been the main sources of methionine in poultry diets. Liquid MHA-FA contains approximately 12% water and 88% MHA molecules, mostly monomers (65%) as well as dimers and trimers (23%). The calcium salt of DLM-HMB has recently been introduced to the feed industry. Methionine hydroxy-analogue calcium salt (MHA-Ca) is a powder containing at least 84% monomeric hydroxy-analogue, 14% calcium and 2% water (Lemme et al., 2007). According to the literature, the average relative bioavailability of MHA products compared to DLM is approximately 75–80% on an equimolar basis (Baker, 2006; Sauer et al., 2008). Whereas L-methionine and D-methionine are actively absorbed (transported against a concentration gradient), MHA is absorbed by the H+ dependent system which is slower than the Na+ system in DL-Met (Maenz and Engele-Schaan, 1996a). Mitchell and Lemme (2008) demonstrated that the use of MHA products in less efficient polymeric forms is the major reason for their lower bioavailability relative to DLM forms. According to
MAENZ and ENGELE-SCHAAN (1996b) and DREW et al. (2003), a substantial portion of MHA is lost due to microbial degradation in the small intestine. Thus, MHA may be less efficiently absorbed and utilised by birds than DL-methionine. Since both excessive and insufficient intake of sulphur amino acids can adversely affect bird performance and meat quality, a number of questions still need to be addressed to make poultry producers and nutritionists feel more confident about efficient use of different methionine sources and levels. Therefore, the aim of this study was to compare the effects of different dietary inclusion levels of MHA-Ca and DLM on the growth performance, carcass yield and meat quality of broiler chickens.

Material and methods

The experiment was carried out in a poultry house in Baldy, owned by University of Warmia and Mazury in Olsztyn, Poland. A total of 891 day-old male Ross 308 chickens were randomly divided into nine treatment groups with 11 replicates of nine birds each and were kept in floor pens. All birds had free access to feed and water. Each pen was equipped with nipple drinkers and a feeder to be manually filled on a daily basis. The heating and light program was consistent with the Ross Broiler Management Manual (AVIAGEN, 2012). The experimental procedure was approved by the Local Animal Experimentation Ethics Committee in Olsztyn (decision no. 51/2012).

The proximate chemical composition of basal starter (0–21 days) and grower/finisher (22–35 days) diets is shown in Table 1. In the starter and grower/finisher periods, the methionine content of basal diets was around 0.30% and 0.27%, respectively. The basal diets were supplemented with four levels of dietary methionine (0.04, 0.08, 0.16, 0.24%) and two different methionine sources (DLM and MHA-Ca) to obtain eight experimental diets: DLM_0.04 (supplemented with 0.04% of DLM), DLM_0.08 (supplemented with 0.08% of DLM), DLM_0.16 (supplemented with 0.16% of DLM), DLM_0.24 (supplemented with 0.24% of DLM), MHA_0.04 (supplemented with 0.04% of MHA-Ca), MHA_0.08 (supplemented with 0.08% of MHA-Ca), MHA_0.16 (supplemented with 0.16% of MHA-Ca), MHA_0.24 (supplemented with 0.24% of MHA-Ca). A negative control (NC) group was also created, in which the diet did not contain any supplemental methionine. The experimental design consisted of nine dietary treatments. Both methionine sources replaced maize and were compared on a weight to weight basis. The nutritional value of all experimental diets corresponded to the nutrient requirements of broiler chickens, except for methionine and methionine+cysteine (SMULIKOWSKA and RUTKOWSKI, 2005). The maximum inclusion levels of the products were determined so as not to exceed excessively the recommended content of standardised ileal digestible methionine+cysteine, i.e. 0.86% and 0.77% in starter and grower diets, respectively. The crude protein and amino acid content of the experimental diets and the content of added amino acids were analysed in the Evonik Laboratory (Hanau, Germany). Diet samples were analysed in duplicate for crude protein using AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS) (2005) method number 976.05. Amino acids were determined by ion-exchange chromatography as described by LLAMES and FONTAINE (1994) and in line with COMMISSION DIRECTIVE (1998). Supplemented MHA-Ca was analysed according to the method proposed by VDLUFA (1997).
Table 1. Composition and nutritional value of starter and grower/finisher basal diets

Zusammensetzung und Nährstoffgehalte der Starter- und Grower/Finisher-Grundrationen

<table>
<thead>
<tr>
<th>Composition, %</th>
<th>Starter</th>
<th>Grower/Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>36.25</td>
<td>31.58</td>
</tr>
<tr>
<td>Soybean meal, 48 CP %</td>
<td>33.22</td>
<td>30.00</td>
</tr>
<tr>
<td>Wheat</td>
<td>23.18</td>
<td>28.15</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.20</td>
<td>6.16</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.70</td>
<td>1.82</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.21</td>
<td>1.17</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>HCl-L-Lysine</td>
<td>0.25</td>
<td>0.18</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin-mineral premix</td>
<td>0.55¹</td>
<td>0.55²</td>
</tr>
<tr>
<td>Nutritional value, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ME (MJ/kg)</td>
<td>12.6</td>
<td>13.4</td>
</tr>
<tr>
<td>Crude protein,%</td>
<td>22.15</td>
<td>19.59</td>
</tr>
<tr>
<td>SID³ Methionine, %</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>SID Methionine+Cysteine, %</td>
<td>0.61</td>
<td>0.54</td>
</tr>
<tr>
<td>SID Lysine, %</td>
<td>1.20</td>
<td>1.04</td>
</tr>
<tr>
<td>SID Threonine, %</td>
<td>0.78</td>
<td>0.68</td>
</tr>
</tbody>
</table>

¹Supplements per kg of starter feed: Vit. A 12000 I. U., Vit. D₃ 5000 I. U., Vit. E 75 mg, Vit. K 4 mg, Vit. B₁ 5 mg, Vit. B₂ 8 mg, Vit. B₆ 5 mg, Vit. B₉ 16 µg, Folic acid 2.0 mg, Biotin 0.2 mg, Choline 400 mg, Pantothenic acid 13 mg, Nicotinic acid 80 mg, Mn 120 mg, Zn 80 mg, I 1.0 mg, Fe 40 mg, Cu 12 mg, Se 0.2 mg.

²Supplements per kg of grower/finisher feed: Vit. A 10000 I. U., Vit. D₃ 5000 I. U., Vit. E 50 mg, Vit. K 3 mg, Vit. B₁ 4 mg, Vit. B₂ 7 mg, Vit. B₆ 4 mg, Vit. B₉ 16 µg, Folic acid 2.0 mg, Biotin 0.18 mg, Choline 350 mg, Pantothenic acid 13 mg, Nicotinic acid 60 mg, Mn 120 mg, Zn 80 mg, I 0.8 mg, Fe 40 mg, Cu 10 mg, Se 0.2 mg.

³SID – standardised ileal digestible.

The body weight of birds (FBW), feed intake (FI) and mortality rates were determined throughout the experiment. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated for each group. At the end of the growth trial a total of 11 birds representing average BW of each group were selected from five experimental groups (NC, DLM₀.₅₈, DLM₀.₂₄, MHA₀.₅₈, MHA₀.₂₄) to measure the effect of methionine supplementation on carcass quality. Carcass data included body weight before slaughter (BWBS), cold carcass weight (CCW, after 24 h of chilling), carcass yield, the proportions of breast meat without skin, thigh meat and drumstick meat and the proportion of abdominal fat, relative to BWBS, were determined according to the method of ZIOLECKI and DORUCHOWSKI (1989).

Dissected breast muscles were forwarded to the Meat Quality Assessment Laboratory at the University of Warmia and Mazury in Olsztyn for a qualitative analysis. The following physicochemical properties of meat were determined: ultimate pH (pHu), colour and water-holding capacity. Prior to pHm and forced drip loss measurements, the samples were three times passed through a mincer (mesh size 2 mm). Minced meat was mixed thoroughly. Acidity was determined based on the pH of the water homogenates of meat (meat to distilled water ratio of 1:1) using a combination Double Pore electrode (Hamilton) and a 340i pH-meter (WTW). Natural drip loss (HONIKEL, 1998), cooking loss (HONIKEL, 1998) and forced drip loss were determined by the GRAU and HAMM method (van OECKEL et al., 1999). Meat colour parameters L*, a*, b* were measured in the CIE LAB system (CIE, 1978), three times, by the reflectance method, with a HunterLab MiniScan XE Plus spectro-colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA), at different points over the muscle cross-section area, with standard illuminant D₆₅ and a 10° standard observer angle. Before each session, the apparatus was standardised using black and white standard plates. Shear force was measured after heat treatment, using a Warner-Bratzler head (500 N, speed 100 mm/min) attached to the INSTRON 5542 universal testing machine. Meat samples were prepared as described by HONIKEL (1998), wrapped in aluminium foil, stored at 4°C for 24 h, and cut into cylinders (three cylinders, 1.27 cm in diameter, 2 cm in height). The maximum force (N) required to shear across the muscle fibres was recorded.
Statistical analyses

The STATISTICA software package version 10.0 (STATSOFT INC., 2011) was used to determine whether variables differed between treatment groups. Two-way ANOVA was performed to assess the effects of the supplementation levels of methionine, the source of methionine and the interaction between the level and source of methionine (source × level) (SNEDECOR and COCHRAN, 1989). When the ANOVA indicated significant treatment effects, means were separated using Duncan’s multiple range test. In the Tables, results are presented as mean values with pooled standard errors. Data were checked for normal distribution before the statistical analysis was performed. Mortality data were transformed (arc sin) and assessed by Duncan’s test. Differences were considered to be significant if \( P \leq 0.05 \). A nonlinear exponential model was applied to estimate the relative efficacy of liquid MHA-FA to DLM. The general linear models procedure of the SAS system (SAS INSTITUTE INC., 2000) was applied, fitting the following nonlinear equation:

\[
y = a + b \times (1 - e^{(c_1 x_1 + c_2 x_2)})
\]

where \( y \) = performance criterion (weight gain, feed conversion, breast meat yield, etc.), \( a \) = intercept (animal 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, \( x_1 \) and \( x_2 \) = dietary levels of pure DLM and liquid MHA-FA, respectively. According to LITTELL et al. (1997), bio-efficacy values for liquid MHA-FA relative to DLM were expressed as the ratios of regression coefficients; \( c_2/c_1 \) = relative bio-efficacy.

Results

The analysed protein and amino acid levels in the experimental diets are shown in Table 2. In general, the analysed crude protein levels and thus all amino acid levels were slightly lower than the intended levels given in Table 1. However, excellent performance values obtained in this experiment suggest that the diets supplied adequate amounts of nutrients. The analysed levels of free methionine, corresponding to supplemental DL-methionine, and the analysed levels of MHA-Ca confirm that methionine was incorporated into and mixed with feed, and the intended supplementation levels were achieved. From a chemical point of view, MHA-Ca is not methionine, which is why the increasing levels of MHA-Ca supplementation were not reflected in the results of total methionine or methionine+cysteine analyses.
Table 2. Analysed amino acid content of experimental diets (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NC</th>
<th>DLM0.04</th>
<th>DLM0.08</th>
<th>DLM0.16</th>
<th>DLM0.24</th>
<th>MHA0.04</th>
<th>MHA0.08</th>
<th>MHA0.16</th>
<th>MHA0.24</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLM</td>
<td>0.00</td>
<td>0.04</td>
<td>0.08</td>
<td>0.16</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MHA</td>
<td>0.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>0.08</td>
<td>0.16</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Starter (0–21 days)**

Crude protein 20.6 20.7 21.0 20.6 20.9 20.4 20.2 20.5 20.2

Methionine 0.29 0.33 0.37 0.45 0.51 0.30 0.29 0.29 0.29

Methionine+Cysteine 0.62 0.67 0.70 0.77 0.84 0.62 0.61 0.61 0.62

Lysine 1.13 1.17 1.17 1.15 1.16 1.16 1.12 1.13 1.14

Threonine 0.80 0.81 0.8 0.79 0.81 0.80 0.77 0.79 0.78

Free methionine – 0.04 0.07 0.15 0.22

MHA-Ca 0.03 0.08 0.16 0.23

**Grower (22–35 days)**

Crude protein 19.01 18.9 19.1 18.9 19.2 19.2 19.0 19.0 19.1

Methionine 0.27 0.31 0.34 0.42 0.50 0.28 0.27 0.28 0.27

Methionine+Cysteine 0.59 0.62 0.62 0.73 0.81 0.59 0.58 0.59 0.59

Lysine 1.05 1.02 1.02 0.99 1.03 1.04 1.03 1.05 1.03

Threonine 0.72 0.71 0.70 0.69 0.72 0.71 0.71 0.72 0.72

Free methionine – 0.04 0.08 0.15 0.22

MHA-Ca 0.04 0.07 0.14 0.21

DLM – DL-methionine, MHA – methionine hydroxy-analogue calcium salt.

Throughout the experiment (0–35 days), mortality rates ranged from 2.02 to 7.07%, but data transformation into arc sin revealed no significant differences between groups (Table 3). Regardless of the dietary methionine source, all experimental groups were characterised by a significantly improvement in FBW, ADG, FI and FCR, compared to the NC treatment \((P\leq 0.001)\). The source and level of methionine had a significant \((P=0.001)\) effect on the ADG and FBW of birds. DL-methionine, especially at high doses, contributed to a significant increase in both parameters, as confirmed by an interaction between the experimental factors \((S\times Le, P=0.005)\). Broiler chickens fed on DLM consumed significantly more feed per day than birds fed on MHA-Ca \((P=0.035)\), but FCR values were similar in all groups, irrespective of the dietary methionine source. FCR improved significantly with increasing methionine levels \((P=0.001)\). The interaction between the experimental factors shows that broilers fed on a diet supplemented with 0.04% MHA-Ca were characterised by the highest feed intake per kg BWG \((S\times Le, P=0.001)\). The performance responses of birds to either methionine source were non-linear, allowing for multi-exponential regression in order to determine the relative bio-availability of MHA-Ca as compared to DL-Met (Figures 1 and 2). The following exponential regression equations were derived for overall body weight gain (0–35 d) and feed conversion ratio (0–35 d): \(Y_{BWG} = 52.0 + 12.7 \times (1 - EXP^{-(24.3 DLM + 11.5 MHA-Ca)})\), \(Y_{FCR} = 1.79 - 0.22 \times (1 - EXP^{-(30.5 DLM + 13.6 MHA-Ca)})\).

Consequently, the biological availability of MHA-Ca relative to DLM was determined at 47% for BWG \((11.5/24.3)\) and 45% for FCR \((13.6/30.5)\) in this weight to weight comparison.
### Table 3. Effects of the source and level of dietary methionine on growth parameters of broiler chickens (n = 99)

<table>
<thead>
<tr>
<th>Group, factor</th>
<th>FBW 35, kg</th>
<th>ADG 0–35, g</th>
<th>FI 0–35, g/day</th>
<th>FCR 0–35, kg/kg DWG</th>
<th>Mortality, arc sin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>1.86</td>
<td>52.0</td>
<td>93.1</td>
<td>1.77</td>
<td>0.009</td>
</tr>
<tr>
<td>DLM0.04</td>
<td>2.15c</td>
<td>60.3c</td>
<td>97.4</td>
<td>1.63b</td>
<td>0.012</td>
</tr>
<tr>
<td>DLM0.08</td>
<td>2.23abc</td>
<td>62.4abc</td>
<td>102</td>
<td>1.61b</td>
<td>0.009</td>
</tr>
<tr>
<td>DLM0.16</td>
<td>2.30ab</td>
<td>64.4ab</td>
<td>102</td>
<td>1.60b</td>
<td>0.009</td>
</tr>
<tr>
<td>DLM0.24</td>
<td>2.30ab</td>
<td>64.5ab</td>
<td>102</td>
<td>1.58b</td>
<td>0.009</td>
</tr>
<tr>
<td>MHA0.04</td>
<td>2.02a</td>
<td>56.6a</td>
<td>99.0</td>
<td>1.75a</td>
<td>0.011</td>
</tr>
<tr>
<td>MHA0.08</td>
<td>2.14ad</td>
<td>60.0ad</td>
<td>96.7</td>
<td>1.62b</td>
<td>0.009</td>
</tr>
<tr>
<td>MHA0.16</td>
<td>2.20bc</td>
<td>61.7bc</td>
<td>97.8</td>
<td>1.59b</td>
<td>0.010</td>
</tr>
<tr>
<td>MHA0.24</td>
<td>2.32a</td>
<td>65.2a</td>
<td>99.4</td>
<td>1.56b</td>
<td>0.012</td>
</tr>
<tr>
<td>SEM</td>
<td>0.018</td>
<td>0.503</td>
<td>0.561</td>
<td>0.010</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Source (S)  
- DLM  
- MHA  

Level (Le)  
0.04  
0.08  
0.16  
0.24  

P-value:  
- NC vs DLM < 0.001 < 0.001 < 0.001 < 0.001  
- NC vs MHA < 0.001 < 0.001 < 0.001  
- Source (S) 0.001 0.001 0.035 0.039 0.694  
- Level (Le) 0.001 0.001 0.236 0.001 0.812  
- S × Le1 0.005 0.005 0.063 0.001 0.153


1Methionine source × level interaction.

Values in a column with no common superscript letter differ significantly; abc – P ≤ 0.05.

Carcass characteristics are presented in Table 4. Dietary supplementation with synthetic methionine contributed to an increase (P≤0.001) in BWBS and, consequently, in CCW, regardless of the amino acid used. A significant (P<0.001) increase was also noted in the breast muscle yield of experimental group chickens. Broiler chickens fed on DL-methionine had significantly (P=0.015) higher final BWBS and, consequently, higher CCW than birds fed on MHA-Ca. Methionine addition at 0.24% contributed to a significant (P=0.001) increase in BWBS and CCW, compared to lower dietary methionine levels. The interaction between the experimental factors revealed that birds fed on a diet supplemented with 0.08% MHA-Ca were characterised by the lowest BWBS and the lowest CCW (S × Le, P=0.001). Breast muscle yield was significantly higher in chickens that received 0.08% and 0.24% DL-methionine and 0.24% MHA-Ca than in those fed on 0.08% MHA-Ca (S × Le, P=0.026). The dressing percentage of broiler carcasses ranged from 69.6% to 72.5%, and it was not affected by the level or source of dietary methionine and neither were thigh and drumstick muscle yields and abdominal fat content.
Dietary methionine levels generally had no significant effect on breast muscle quality (Table 5) except for colour parameter a*, which suggested an interaction with methionine source \( (P=0.026) \). The highest a* values were noted in the breast muscles of birds fed on 0.08% MHA-methionine and 0.24% DL-methionine, at 6.99 and 6.86, respectively. \( pH_u \) values varied depending on methionine source \( (P=0.021) \). Neither the source nor the level of dietary methionine significantly affected the water-holding capacity of breast muscles. The obtained results indicate that the breast muscles of chickens fed on a diet with 0.08% and 0.24% MHA were characterised by better water-holding capacity, i.e. higher ability to hold their own and added water. Average natural drip loss reached 2.55% in chickens fed on 0.24% MHA-methionine. The muscles of broilers fed on a diet supplemented with 0.08% MHA had the lowest natural drip loss (1.69%). The source and level of dietary methionine had no significant effect on the maximum shear force of cooked breast muscle samples. However, the meat of birds fed on supplemental methionine, regardless of its level and source, was characterised by significantly lower values of shear force (higher tenderness), compared to the control group. The lowest value of shear force, at 8.31 N, was noted in the group fed on a diet supplemented with 0.24% DL-methionine.
Table 5. Effects of the source and level of dietary methionine on the physicochemical properties and shear force value of breast muscles (n = 55)

<table>
<thead>
<tr>
<th>Group, factor</th>
<th>pHu</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Drip loss, %</th>
<th>Cooking loss, %</th>
<th>Water-holding capacity, cm²</th>
<th>Shear force, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>5.9</td>
<td>63.4</td>
<td>6.50</td>
<td>14.7</td>
<td>2.43</td>
<td>29.2</td>
<td>3.19</td>
<td>11.9</td>
</tr>
<tr>
<td>DLM 0.08</td>
<td>5.9</td>
<td>62.5</td>
<td>6.27b</td>
<td>14.4</td>
<td>2.28</td>
<td>30.5</td>
<td>2.91</td>
<td>9.12</td>
</tr>
<tr>
<td>DLM 0.24</td>
<td>5.9</td>
<td>62.1</td>
<td>6.86a</td>
<td>14.8</td>
<td>2.28</td>
<td>28.4</td>
<td>2.08</td>
<td>8.31</td>
</tr>
<tr>
<td>MHA 0.08</td>
<td>6.0</td>
<td>61.3</td>
<td>6.99a</td>
<td>14.3</td>
<td>1.69</td>
<td>30.5</td>
<td>2.02</td>
<td>9.02</td>
</tr>
<tr>
<td>MHA 0.24</td>
<td>5.9</td>
<td>61.8</td>
<td>6.35b</td>
<td>14.8</td>
<td>2.55</td>
<td>31.1</td>
<td>2.03</td>
<td>9.03</td>
</tr>
<tr>
<td>SEM</td>
<td>0.014</td>
<td>0.272</td>
<td>0.125</td>
<td>0.171</td>
<td>0.149</td>
<td>0.506</td>
<td>0.220</td>
<td>0.273</td>
</tr>
</tbody>
</table>

Source (S)

| DLM | 5.9b| 62.3| 6.54| 14.6| 2.29         | 29.4           | 2.49                     | 8.71           |
| MHA | 6.0a| 61.6| 6.67| 14.6| 2.12         | 30.8           | 2.03                     | 9.02           |

Level (Le)

| 0.08 | 5.9 | 61.9| 6.60| 14.4| 1.99         | 30.5           | 2.46                     | 9.07           |
| 0.24 | 5.9 | 62.0| 6.60| 14.8| 2.42         | 29.8           | 2.06                     | 8.67           |

P-value:

| NC vs. DLM | 0.462| 0.136| 0.909| 0.751| 0.718        | 0.854           | 0.247                    | < 0.001        |
| NC vs. MHA | 0.214| 0.015| 0.606| 0.705| 0.441        | 0.249           | 0.061                    | < 0.001        |
| Source (S) | 0.021| 0.241| 0.630| 0.937| 0.635        | 0.193           | 0.344                    | 0.484          |
| Level      | 0.503| 0.973| 0.995| 0.264| 0.235        | 0.499           | 0.407                    | 0.368          |
| S × Le³    | 0.919| 0.472| 0.026| 0.866| 0.233        | 0.196           | 0.391                    | 0.355          |

DLM – DL-methionine, MHA – methionine hydroxy-analogue calcium salt.

1Methionine source × level interaction.

Values in a column with no common superscript letter differ significantly; abc – P ≤ 0.05.

Discussion

The results of the present study suggest that increasing levels of dietary methionine improve the ADG and FBW of broiler chickens. In comparison to the control group where the FBW of broilers reached 1.86 kg, the highest inclusion levels of either methionine source improved bird performance by approximately 24%, which indicates that the basal diet was deficient in sulphur amino acids. The highest methionine doses improved also the FCR by 10%, compared to the control diet, thus increasing the efficiency of broiler production. In view of the FBW and FCR of 2.250 kg and 1.566 kg/kg, respectively, recommended for 35-day-old male Ross 308 broilers by the breeder company AVIAGEN (2012), the FBW and FCR values achieved in the present study at the highest inclusion levels of either product can be considered excellent. The effect of increased methionine intake on the FBW of broiler chickens, observed in the present experiment, is consistent with the findings of other authors who reported that the addition of different sources of methionine above the recommended requirements of broilers improves their performance in terms of body weight gain and feed conversion efficiency (PILLAI et al., 2006; AHMED and ABBAS, 2011). In a study of turkeys, LEMME et al. (2005) also demonstrated that birds fed on diets with the highest level of methionine+cysteine were characterised by the highest body weight gain. CAFÉ and WALDRROUP (2006) found that increasing methionine levels above the NRC (1994) recommendations significantly improved the BW of broiler chickens only at 35 days of age. No significant differences in BW were observed in older birds, which may indicate that their response to dietary amino acids decreased with age.

In the present study, high FBW of broiler chickens supplemented with methionine contributed to increased carcass weight. Birds receiving higher methionine doses were also characterised by a higher breast muscle yield, whereas the yields of other muscles were comparable in all groups. The present results corroborate the findings of other authors.
who demonstrated that increased supply of methionine, regardless of its source, improved breast meat weight and yield (Estévez-Garcia and Mack, 2000; Lemme et al., 2007) and had no effect on abdominal fat percentage (Estévez-Garcia and Mack, 2000). According to Ahmed and Abbas (2011) and Wallis (1999), increasing dietary levels of methionine significantly decrease abdominal fat content in broiler chickens. Hickling et al. (1990) suggested that improved breast meat yield in response to increasing dietary methionine and lysine levels could result in economic benefits depending on the cost of amino acid supplementation and the price of poultry meat.

In the present experiment, the source of dietary methionine had a significant effect on the FBW and FCR of broiler chickens in corresponding treatments, particularly at the 0.04% inclusion level. Broilers fed on diets supplemented with 0.16% DL-methionine and 0.24% MHA-Ca were characterised by similar FBW and FCR. Thus, multiple range tests would suggest no differences between methionine sources except for the lowest supplementation level. However, multi-exponential regression revealed MHA-Ca to be only 47% and 45% as efficient as DLM with respect to BWG and FCR. The above values are lower than that reported by Elwert et al. (2008) who found an average bioavailability of 63% in two feeding trials with MHA-Ca. However, in the second experiment performed by Elwert et al. (2008), the relative bioavailability of MHA-Ca compared to DLM was only 30% for FBW and 59% for feed efficiency in 42-day-old broilers. Dilger and Baker (2008) determined an average bioavailability of 66% for MHA-Ca as compared to DLM in two broiler growth assays. Thus, the finding of the current study confirm that MHA-Ca bioavailability is clearly lower than DLM bioavailability, which indicates that in this trial DLM would be needed at only 45–47% of the amount of MHA-Ca to achieve the same performance.

Despite lower daily feed intake, chickens that received the methionine hydroxy-analogue consumed the same amount of feed per kg of BWG as birds fed on DLM, which points to a lower growth rate. In two choice-feeding experiments by Lemme et al. (2008) and Paulicks et al. (2009), respectively, broilers had a slightly greater preference for diets supplemented with DLM as compared to those supplemented with liquid MHA-FA. Thus, reduced feed intake in MHA-Ca treatments observed in the present trial could be related to the taste, palatability and other parameters of diet attractiveness.

Similar to body weight and FCR, also breast meat yield was lower in birds fed on MHA-Ca, compared to DLM, at lower inclusion levels. Meirelles et al. (2003) did not note significant differences between the sources of methionine (DLM and MHA-FA) with respect to carcass yield, breast and leg muscle yields and abdominal fat content. In contrast, in two experiments with broiler chickens conducted by Elwert et al. (2008), MHA-Ca was found to be only 53% and 55% as efficient as DL-Met in achieving the same breast meat yield (% of carcass), which suggests that breast muscle growth is very sensitive to adequate methionine concentration in the feed. Also Payne et al. (2006) demonstrated that broilers fed on DL-methionine were more effective at depositing leg meat and breast meat, which resulted in higher breast meat yield, than those fed on liquid MHA-FA. As expected, DL-methionine was a more effective amino acid source than MHA-Ca administered in the same amount, most likely due to the higher availability of the former. Some research has shown, using different methodologies, that the average relative bioavailability of MHA compared to DLM in various animal species ranges from 65% to 95%, and that chickens fed on an MHA-supplemented diet are characterised by lower growth performance than those fed on a DLM-supplemented diet (Vazquez-Anon et al., 2006; Sauer et al., 2008). It should be noted that Vazquez-Anon et al. (2006) used multiple regression for a meta-analysis, whereas Sauer et al. (2008) relied on multi-exponential regression analysis. Sauer et al. (2008) confirmed the method to be valid as the same performance could be achieved with both products (in contrast to the method proposed by Vazquez-Anon). Elwert et al. (2008), Lemme et al. (2002), and Hoehler et al. (2005) also provided sound validation for the above methodology. The latter researchers included a third product, diluted DLM, in their multi-regression approaches. DLM was diluted with sugar or starch to 65% purity, and the bioavailability of diluted DLM was close to the expected value of 65%. According to Elwert et al. (2008), the bioefficacy of MHA-Ca is not different from that of liquid MHA-FA when compared on “as is” basis, whereas on a molar basis, liquid MHA-FA is 73% as efficient and MHA-Ca is 77% as efficient as DLM. The above could be due to differences in the purity of the products (MHA-FA ~ 88%, MHA-Ca ~ 84%) and physiological factors since MHA-Ca does not contain dimers and trimers which are poorly available (Mitchell and Lemme, 2008). Differences in relative bioefficacy may be explained by reduced intestinal absorption of MHA as compared to DLM, inefficient conversion of MHA to methionine after absorption or a combination of both factors (Maenz and Engele-Schaan, 1996a,b; Drew et al., 2003, Mitchell and Lemme, 2008). According to Ahmed and Abbas (2011), broiler chicks fed on diets with methionine content above the National Research Council (1994) recommendations were characterised by a significant decrease in abdominal fat content. Methionine may affect lipid metabolism by simulating the oxidative catabolism of fatty acids via its role in
carnitine synthesis, thus offering a potential for reduced carcass fatness in commercial production (SCHUTTE et al., 1997).

We analysed the following technological properties of meat: acidity (pH), colour, natural drip loss, forced drip loss and cooking loss. Meat pH, affected by post-mortem glycolysis taking place in muscle tissue, has a profound influence on quality since it determines traits responsible for the processing suitability, eating attributes and shelf-life of meat (WOELFEL et al., 2002). At slaughter, the pH of muscle tissue is close to neutral, and it decreases post mortem due to acidification. Post-mortem pH decline is one of the most important events in the conversion of muscle to meat due to its impact on meat texture, colour and water-holding capacity (FANATICO et al., 2007). In all analysed muscles, colour lightness ($L^*$) was higher than that reported by other authors (WOELFEL et al., 2002; JAKUBOWSKA et al., 2004), most likely due to a high contribution of the $b^*$ component, which increases significantly in the muscles of chickens fed on diets supplemented with synthetic methionine (JIAO et al., 2010). According to GARDZIELEWSKA et al. (2005), DL-methionine added to diets for turkey hens at 0.13 and 0.24% led to minor changes in the physicochemical properties of breast muscles, including a decrease in acidity (5.70 and 5.76), a darker colour ($L^*$=55.22 and 54.35) due to an increase in redness ($a^*$=5.18 and 5.41) and better water-holding capacity, in comparison with the breast muscles of control group birds. In our study, the pH of breast muscles was slightly higher in turkeys fed on DLM than in birds fed on MHA-Ca. Similarly as in the cited experiment, the contribution of redness increased only in the muscles of turkeys that received diets supplemented with higher DLM levels. WANG et al. (2009), who investigated dietary supplementation with DLM in laying hens, also reported a considerable effect of methionine on the lightness and redness of breast muscles. Methionine supplementation at 4.0 g/kg feed increased colour lightness, whereas 5.4 g of methionine per kg significantly increased $a^*$ value (2.98) compared to that of 3.2 g/kg ($a^*$=2.18) and 4.0 g/kg methionine ($a^*$=1.91). In the present study, neither the source nor the level of dietary methionine affected the natural drip loss, cooking loss, tenderness and water-holding capacity of breast muscles, i.e. the ability of muscle proteins to absorb water and hold it during heat treatment. The values of all parameters were typical of high-quality meat.

Conclusions
Higher dietary methionine levels improved the growth performance and breast muscle yield of broiler chickens, but they had no influence on meat quality, except for improvement in tenderness (lower shear force values). DLM was a more effective amino acid source than MHA-Ca, and it contributed to higher production efficiency. The source of dietary methionine had no effect on carcass yield or breast muscle quality.

Summary
The present study investigated the effect of different dietary levels of DL-methionine (DLM) and MHA-Ca on broiler chicken performance, carcass characteristics and meat quality. A total of 891 day-old male Ross 308 chickens were allocated to nine groups with 11 replicates each, and were fed on diets supplemented with four levels of dietary methionine (0.04, 0.08, 0.16, 0.24%) and two different methionine sources (DLM and MHA-Ca). The negative control (NC) diet did not contain any supplemental methionine.

Dietary treatments had a significant effect on the average daily gain (ADG) and final body weight (FBW) of birds ($P<0.001$), which were significantly higher in groups given high doses of DLM. Feed conversion ratio (FCR) improved significantly with increasing methionine levels ($P<0.001$). Methionine addition at 0.24% contributed to a significant ($P<0.001$) increase in body weight and carcass weight, compared to lower dietary methionine levels. A higher breast muscle yield ($P=0.004$) was noted in chickens that received diets supplemented with higher amino acid levels. The source of dietary methionine had no effect on carcass characteristics, however, an interaction between the experimental factors showed that birds fed on 0.08% MHA-Ca were characterised by the lowest body weight before slaughter (BWBS), cold carcass weight (CCW) ($P=0.001$) and breast muscle yield ($P=0.026$). Dietary methionine levels had no significant effect on breast muscle quality, whereas the breast muscles of chickens fed on diets supplemented with DLM had lower pH$_b$ compared to those fed on MHA-Ca diets ($P=0.021$). In conclusion, increasing inclusion levels of methionine improved the growth performance and breast muscle yield of broiler chickens, but they had no influence on meat quality. DLM was found to be a more effective amino acid source than MHA-Ca, and it contributed to higher production efficiency without compromising carcass yield or breast muscle quality.
Key words
Broiler, nutrition, methionine, butanoic acid, growth, carcass quality, meat quality

Zusammenfassung
Einfluss unterschiedlicher Zulagen von DL-Methionin und dem Kalziumsalz von DL-2-Hydroxy-4-[Methyl]-Buttersäure zum Futter auf Wachstum, Schlachtkörper- und Fleischqualität bei Broilern

In der vorliegenden Studie wurde der Einfluss des Zusatzes von DL-Methionin (DLM) und MHA-Ca in unterschiedlicher Höhe zum Futter auf die Leistung, die Schlachtkörpermerkmale und die Fleischqualität von Broilern untersucht. Hierzu wurden 891 männliche Eintagsküken der Herkunft Ross 308 auf 9 Versuchsgruppen mit jeweils 11 Wiederholungen verteilt. Die beiden Methioninquellen (DLM, MHA-Ca) wurden jeweils in vier Stufen zugesetzt (0,04, 0,08, 0,16, 0,24%). Die negative Kontrolle (NC) enthielt kein zugesetztes Methionin.

Die Methioninzulage hatte einen signifikanten Effekt auf die durchschnittlichen, täglichen Zunahmen (ADG) und das Mastendgewicht (FBW) der Broiler (P = 0,001). Die Effekte waren für das DLM deutlicher als für das MHA-Ca. In ähnlicher Weise verbesserte sich die Futterverwertung (FCR) signifikant mit der Methioninzulage (P = 0,001). Im Vergleich zu den niedrigeren Zulagestufen erhöhte die Zulage von 0,24% Methionin das Körper- und das Schlachtgewicht signifikant (P = 0,001). In ähnlicher Weise war der Brustmuskelanteil bei höheren Methioninzulagen höher als bei niedrigeren (P = 0,004). Die Methioninquelle hatte keinen Einfluss auf die Schlachtkörpermerkmale, allerdings lag eine signifikante Interaktion zwischen der Methioninquelle und der Methioninzulagenhöhe vor. Die Futtergruppe mit 0,08% MHA-Ca hatte das geringste Lebendgewicht vor der Schlachtung (BWBS), das geringste Schlachtkörpargewicht kalt (CCW; P = 0,001) und den geringsten Brustfleischanteil (P = 0,026). Die Methioninzulage hatte dagegen keinen Einfluss auf die Qualität des Brustfleischs, allerdings war der pHu bei DLM signifikant geringer als bei MHA-Ca (P = 0,021). Es wurde der Schluss gezogen, dass mit der Höhe der Methioninhalte im Futter die Wachstumsleistung und der Brustfleischanteil erhöht werden, ohne sich auf die Fleischqualität auszuwirken. Dabei erwies sich DLM effektiver als MHA-Ca. DLM bewirkte eine bessere Produktionseffizienz ohne nachteilige Effekte auf die Schlagtausbeute oder die Brustfleischqualität.

Stichworte
Broiler, Fütterung, Methionin, Buttersäure, Wachstum, Schlachtkörperqualität, Fleischqualität

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