

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Efficacy of dimethylglycine as a feed additive to improve broiler production

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/156896> since 2015-12-14T13:35:34Z

Published version:

DOI:10.1016/j.livsci.2014.03.003

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Livestock Science (2014) 164: 81–86**

2

3

Efficacy of dimethylglycine as a feed additive to improve broiler production.

4

5

6 I.D. Kalmar, M.W.A. Verstege, D. Vanrompay, K. Maenner, J. Zentek, C. Iben, R. Leitgeb,

7

A. Schiavone, L. Prola, G.P.J. Janssens

8

9 **Efficacy of dimethylglycine as a feed additive to improve broiler production.**

10

11

12 I.D. Kalmar ^{a,*,1}, M.W.A. Verstegen ^c, D. Vanrompay ^b, K. Maenner ^d, J. Zentek ^d, C. Iben ^e,
13 R. Leitgeb ^{e,f}, A. Schiavone ^g, L. Prola ^g, G.P.J. Janssens ^a

14

15

16 ^a Laboratory of Animal Nutrition, Ghent University, Belgium, 9820 Merelbeke, Belgium

17 ^b Department of Molecular Biotechnology, Ghent University, 9000 Gent, Belgium

18 ^c Animal Nutrition Group, Wageningen University, 6700 Wageningen, the Netherlands

19 ^d Institute of Animal Nutrition, Free University of Berlin, 14195 Berlin, Germany

20 ^e Institute of Nutrition, Veterinary University of Vienna, 1210 Vienna, Austria

21 ^f Poultry trial station, 9311 Kraig, Austria

22 ^g Department of Veterinary Science, University of Turin, 10095 Grugliasco, Italy

23

24 * Corresponding author. Tel.: Tel: +32 9 264 75 27; Fax: +32 9 264 62 19.

25 *E-mail address:* nutrition@ugent.be (I. Kalmar).

26 ¹ Present address: Laboratory of Animal Nutrition, Ghent University, Belgium, Heidestraat

27 19, 9820 Merelbeke, Belgium

28

29 **ABSTRACT**

30 Dimethylglycine (DMG) is a naturally occurring glycine derivative, which is useful as
31 additive to broiler diets as it improves nutrient digestibility and reduces the development of
32 broiler ascites syndrome. This study evaluated the efficacy of dietary DMG to enhance
33 performance of broiler chickens. Three trials were conducted to evaluate the effect of dietary
34 supplementation with 1 g Na DMG/kg on growth performance and carcass characteristics. In
35 Trial 1, the effect of sex was also assessed in a 2 x 2 factorial arrangement of treatments. In
36 Trials 1 (Germany), 2 (Austria), and 3 (Italy), each treatment consisted of 6, 12, and 11
37 replicate pens with 20, 15, and 16 one-day-old broiler chickens per pen, respectively. Dietary
38 DMG supplementation resulted in improved feed conversion ratio (FCR) in the starter phase
39 by 8.8 ($P = 0.004$), 6.4 ($P = 0.001$), and 4.8% ($P = 0.006$) compared with the control diet in
40 Trials 1, 2, and 3, respectively. The overall FCR improved in broiler chickens fed the diets
41 supplemented with DMG by 3.8 and 4.1% in Trials 1 ($P = 0.007$) and 3 ($P = 0.006$),
42 respectively. In addition, final body weight increased by 5.5% ($P = 0.001$) in Trial 2 and
43 production value improved by 6.8% ($P = 0.015$) in Trial 1 by dietary DMG supplementation.
44 Mortality in all trials was similar between dietary treatments. In all 3 trials, cold carcass
45 weight and total meat yield were as well similar between broiler chickens fed the control and
46 DMG diets. In Trial 1, dietary DMG had no effect on breast meat yield in male broiler
47 chickens, but it increased breast meat yield in female broiler chickens (diet x sex, $P = 0.004$).
48 Organoleptic quality of roasted breast meat assessed only in Trial 2 was not affected by
49 dietary treatments. In conclusion, dietary supplementation of DMG at 1 g Na DMG/kg can
50 considerably improve s production performance in broiler chickens.

51 *Keywords:* Broiler; Dimethylglycine; Growth performance ; Feed efficiency

52

53 **1. Introduction**

54 Dimethylglycine (DMG) is a naturally occurring tertiary amino acid in the intermediary
55 metabolism of betaine in living organisms. Dietary supplementation in broiler diets results in
56 improved apparent faecal digestibility of the crude protein and carbohydrate fraction. This is
57 hypothesized to result from an emulsifying effect of DMG in the intestinal tract, which allows
58 non-fat nutrients to be more efficiently absorbed, rendering more nutrients available for
59 utilization (Kalmar et al., 2010; Prola et al., 2013). Dietary DMG has also been shown to
60 improve carcass characteristics by decreasing fat deposition and increasing meat yield. These
61 changes are linear in the range between 0 and 1 g Na DMG/kg feed and are more pronounced
62 with increased level of dietary polyunsaturated fatty acids (Kalmar et al., 2011). Kalmar et al.
63 (2011) suggested enhanced utilisation of dietary fat as an energy source as a possible
64 underlying basis. Namely, dietary fat is utilised as a source of energy, instead of being
65 deposited as body fat. Consequently, less protein is used to provide energy, which promotes
66 lean growth. Therefore, dietary DMG not only reduces feed costs, but also has potential
67 environmental benefits because of improved protein utilization, which has been demonstrated
68 by reduced N excretion into urine (Kalmar et al., 2010). Possibly, DMG also influences
69 hepatic gene expression by affecting DNA methylation, as has been demonstrated for other
70 methylamine derivatives (Emmert et al., 1996; Niculescu et al., 2006). Effects of dietary DMG
71 on hepatic gene expression are currently under investigation (T. Erkens et al., unpublished
72 data).

73 The aim of this study was to assess the efficacy of dietary supplementation with DMG at a
74 level of 1 g Na DMG/kg to improve broiler performance. Three broiler trials were conducted
75 at different European locations, at which distinct broiler strains and basal diets were used.

76

77 **2. Materials and methods**

78 *2.1. Experimental design and treatments*

79 Three broiler trials were conducted at different European locations. Trial 1 was conducted
80 at the Free University of Berlin (Berlin, Germany). Trial 2 was conducted at the poultry trial
81 station in Äussere Wimitz (Kraig, Austria). Trial 3 was conducted at the certified (ISO 9001)
82 poultry farm, "Luca Fornello" in Settimo (Torinese, Italy). In each trial, 1-d-old broiler
83 chickens were randomly allocated to pens and fed control, basal diets or basal diets
84 supplemented with 1 g Na DMG/kg. In all trials, feed was offered *ad libitum*.

85 2.1. Animals and management

86 Housing conditions were in all trials in compliance with the minimal space restrictions
87 according to the revised European Treaties series No. 123 (ETS 123). Ingredient, and energy
88 and nutrient composition of basal diets are presented in Tables 1 and 2, respectively.

89 2.1.1. Trial 1

90 A total of 480 one-day old broiler chickens (Cobb Germany Avimex GmbH, Regenstauf,
91 Germany) were randomly assigned to 12 pens with 20 females and 12 pens with 20 males,
92 and reared until 39 d of age. Pens were randomly assigned within sex to 2 dietary treatments
93 with 6 replicate male pens and 6 replicate female pens per treatment. A 3-phase feeding
94 program was used with a starter diet from d 1 until d 14, a grower diet from d 15 until d 28,
95 and a finisher diet from d 29 until d 39. Each floor pen was 2.2 x 1.8 m (length x width) and
96 had softwood shaving litter as bedding. Lighting schedule was 24 h light during the first 3 d,
97 followed by 23 h light:1 h darkness until d 7, and then 18 h light:6 h darkness until slaughter.
98 Ambient temperature was kept at 28°C during the first 2 wk, and after d 15, it was reduced by
99 0.5°C per day until 22°C was reached. Additionally, the temperature at the surface of the
100 bedding was monitored and maintained at about 34°C by infra-red heaters until d 21. Relative
101 humidity was $60.0 \pm 3.5\%$. All birds were vaccinated against coccidiosis (Paracox; Essex
102 Pharma GmbH, Munich, Germany) at 9 d of age by individual oral application at the dose
103 level of 0.1 mL/broiler chicken.

104 *2.1.2. Trial 2*

105 A total of 360 one-day old Ross 308 broiler chickens were randomly allocated in 24 pens
106 of 15 unsexed chickens and reared until 36 d of age. A three-phase feeding program was used
107 with a starter diet from d 1 until d 14, a grower diet from d 15 until d 28, and a finisher diet
108 from d 29 until d 36. Each floor pen was 2.0 x 1.5 m (length x width), and had wood shavings
109 as litter. Lighting schedule was 24 h light during the first 3 d, followed by 22 h light:2 h
110 darkness until slaughter. Ambient temperature was initially kept at 28°C and gradually
111 reduced to 20°C.

112 *2.1.3. Trial 3*

113 A total of 352 one-day old Ross 508 broiler chickens were randomly allocated in 22 pens
114 of 16 birds of both sexes (8 males and 8 females) per pen, and reared for 35 d. A 2-phase
115 feeding program was used with a starter/grower diet from d 1 until d 21 and a finisher diet
116 from d 22 until d 35. Each pen was 1.5 x 1.0 m (length x width), and had rice hulls as litter.
117 Lighting schedule was 23 h light:1 h darkness until d 7 and 18 h light:6 h darkness until
118 slaughter. Infrared lamps were used for heating during the first 3 wk. Minimum and
119 maximum temperatures were 21.9 and 30.4°C in the starter-grower period and 22.4 and
120 26.3°C in the finisher period. At hatching, chicks were vaccinated against coccidiosis,
121 Newcastle disease, and infectious bronchitis (Izovac I.B. H120; Izo S.p.A., Brescia, Italy).
122 The vaccine against coccidiosis was administered in the drinking water, while those for
123 Newcastle disease and infectious bronchitis were administered by inhalation.

124 *2.2. Assessed variables*

125 Body weight (BW) and feed remainders were recorded at the beginning and end of all
126 feeding phases. Mortality was recorded daily. Average daily gain (ADG), average daily feed
127 intake (ADFI), and feed conversion ratio (FCR) were calculated for each feeding phase.

128 Production value (PV) were calculated as follows: $PV = [100 - \text{mortality (\%)} \times \text{BW}$
129 $(\text{g})]/[\text{rearing period (d)} \times \text{FCR} \times 10]$.

130 At slaughter age, 1 broiler chicken per pen in Trial 1 and 1 female and 1 male broiler
131 chickens per pen in Trials 2 and Trial 3 were randomly chosen and humanely euthanised after
132 an 8-h fasting period. In Trial 1 and 2, broiler chickens were euthanised by concussion
133 followed by exsanguination. In Trial 3, broiler chickens were euthanised by individual CO₂
134 gassing followed by exsanguination. In all trials, live weight with empty crop was determined
135 immediately prior to euthanasia. Then, broiler chickens were mechanically plucked after
136 immersion in hot water, manually eviscerated, and weight of abdominal depot fat measured.
137 The remaining carcass was chilled for 24 h at 3°C. Head, neck, and feet at hock joint were
138 removed from chilled carcasses to determine cold carcass weight. Breast meat, legs, and
139 wings were manually dissected to assess meat yield.

140 In Trial 2, organoleptic quality of breast meat was determined using 12 males (1/pen from
141 12 pens) and 12 females (1/pen from 12 pens) per treatment. Pieces of breast meat (3 x 3 x 1
142 cm) were roasted on both sides for 6 min at 180°C and then graded by a taste panel consisting
143 of 4 independent, trained individuals. The meat was subjectively graded for tenderness,
144 juiciness, and taste using scores ranging from 1 to 6.

145 *2.3 Statistical analyses*

146 Data on growth performance were statistically analysed with data per pen as the
147 experimental unit, whereas euthanized birds were used as the experimental unit for carcass
148 characteristics. Normality and homogeneity were tested with the Kolmogorov-Smirnov and
149 modified Levine test, respectively. All traits, except mortality, were analysed using one-way
150 ANOVA. Growth performance data in Trial 1 were analysed with diet, sex, and interactions
151 as independent variables, whereas in Trials 2 and 3, diet was used as an independent variable.
152 Carcass characteristics in all trials were analysed with diet, sex, and interactions as

153 independent variables. Results of the organoleptic test were subject to the general linear
154 model repeated measures analysis of variance with an individual taste panellist as within-
155 subject variable and diet as between-subject variable. Mortality was not normally distributed,
156 hence these data were analysed with the non-parametrical two-way Wilcoxon test with diet as
157 grouping variable. Average values are expressed as means \pm standard error of the means
158 (SEM). All statistics were done in S-plus 8.0 (TIBCO Software Inc., Palo Alto, CA) and
159 SPSS 16.0 (SPSS Inc., Chicago, IL).

160

161 **3. Results**

162 Dietary supplementation with DMG improved FCR during the starter phase in all trials
163 (Tables 3 and 4). Compared to the control, FCR during the starter phase was reduced by 8.8
164 (5.7% for females and 11.8% for males; $P = 0.004$), 6.4% ($P = 0.001$), and 4.8% ($P = 0.006$)
165 in Trial 1, 2, and 3, respectively. In Trial 3, FCR during the finisher phase was 3.6% lower in
166 DMG supplemented broilers ($P = 0.036$). The overall FCR was improved by 3.8% in Trial 1
167 (4.0% for females and 3.6% for males; $P = 0.007$) and 4.1% in Trial 3 ($P = 0.006$). Production
168 value in Trial 1 was 25 units or 6.8% greater in broiler chickens supplemented with DMG (25
169 units or 6.7% for females and 26 units or 6.9% for males; $P = 0.015$). There was no diet x sex
170 interaction on growth performance in Trial 1. In Trial 2, dietary DMG resulted in an increase
171 in both ADFI (3.9%, $P = 0.002$) and ADG (7.0%, $P = 0.001$), and final BW increased by
172 5.5% ($P = 0.001$). In Trial 3, dietary DMG reduced ADFI by 3.7% ($P = 0.027$), but ADG and
173 final BW were similar compared to the control.

174 In Trial 1, dietary DMG had no effect on breast meat yield in male broiler chickens, but it
175 increased breast meat yield in female broiler chickens (diet x sex, $P = 0.004$; Table 3). There
176 was no effect of dietary DMG on carcass characteristics in Trial 2 (Table 4). In Trial 3,
177 abdominal depot fat was 0.3% lower ($P = 0.002$) in broiler chickens fed the DMG diets than

178 those fed the control diets. This small decrease in fat deposition resulted in a 24.7% greater
179 meat yield to abdominal fat ratio ($P = 0.013$). Tenderness, juiciness, and taste of roasted
180 breast meat assessed in Trial 2 were comparable between treatment groups (Table 5).

181

182 **4. Discussion**

183 Feed conversion ratio in the control groups varied among trial sites, i.e., outstanding in
184 Trial 1 (FCR = 1.55 for males and 1.52 for females), satisfactory in Trial 3 (FCR = 1.70), and
185 rather inefficient in Trial 2 (FCR = 1.81). Still, FCR was improved by DMG in Trial 1 and
186 Trial 3. In Trial 2, FCR was only improved during the starter phase. These data indicate a
187 beneficial effect of dietary DMG on FCR over a wide range of broiler chickens, with growth
188 performance being influenced by broiler strains, basal diets, and rearing conditions. Overall,
189 the effect of dietary DMG on FCR was greatest and most consistently present during the
190 starter phase. The underlying mechanism of improved feed efficiency is likely to be, at least
191 partly, the result of improved digestibility of protein and N-free extract because of the
192 emulsifying action of DMG at the intestinal tract (Kalmar et al., 2010; Prola et al., 2013). The
193 indirect effect of increased fat emulsification on improved digestibility of non-fat fractions
194 can be explained by enhanced accessibility for digestive enzymes (Kalmar et al., 2011). Apart
195 from yolk utilization, for which the importance of pancreatic and biliary secretions seems to
196 be negligible, the digestive capacity of fat in broilers increases with age (Freeman, 1976;
197 Krogdahl, 1985). In particular, digestion of vegetable oils, which were the sole or main fat
198 source in current trials, is underdeveloped in broiler chicks until the first 2 wk of age
199 (Freeman, 1976). Therefore, an emulsifying agent is indeed expected to be most efficient in
200 improving digestibility in the broiler chicken during the starter phase. Furthermore, DMG also
201 acts as a glycine precursor (Craig, 2004), and, consequently, leads to the improvement of
202 protein biosynthesis in chicks, where this amino acid is essential (Klasing, 2000). The fact

203 that highest effects of DMG on FCR are consistently noticed in the starter period is thus not
204 surprising.

205 Although sample size was rather limited, an important increase in the ratio between meat
206 yield and abdominal fat was observed in Trial 3. This implies enhanced lean growth in broiler
207 chickens fed the diets supplemented with DMG. Fat accretion has a greater energetic cost per
208 mass unit compared to lean accretion (protein plus water). Thus, an increase in meat to fat
209 ratio also contributes to a more efficient feed conversion. These results are in agreement with
210 previous data, in which a linear inverse relation was observed between abdominal fat pad and
211 dietary DMG supplementation with a range of 0 to 1 g Na DMG/kg feed (Kalmar et al.,
212 2011). A plausible cause of lower fat deposition relative to lean growth in DMG
213 supplemented broilers can be an increase in protein supply as a result of its increased
214 digestibility. This agrees with results of, for instance, Namroud et al. (2008). Those authors
215 showed a decrease in abdominal fat deposition and a concomitant lower FCR in broiler
216 chickens when increasing dietary protein content from 17 to 21%. This is within the range of
217 protein content of current finisher diets. Abdominal depot fat in the control groups of current
218 investigation was also inversely related to protein content of finisher diets. In contrast to
219 Namroud et al. (2008), in which the degree of improvement in FCR was greatest when
220 increasing dietary protein content from 17 to 19% compared to an increase from 19 to 21%,
221 the lowest improvement in FCR on account of DMG was observed at lowest dietary protein
222 content of finisher diet in the current trials. Hence, additional factors are likely to be involved
223 in the working mechanism of DMG.

224 **5. Conclusion**

225 Three feeding trials were conducted at different locations. Although FCR varied widely
226 among trial sites, supplementation with DMG at a dose of 1 g Na DMG/kg resulted in an
227 improvement of feed efficiency during, at least, the starter phase in all trials. Although effects

228 on FCR were most consistently observed and most pronounced during the starter phase, FCR
229 was in 2 of the 3 trials improved over the whole rearing period. In addition, finishing BW and
230 PV were increased in 1 of the 3 trials. Organoleptic quality of roasted breast meat was similar
231 between control and DMG groups. On the whole, this investigation demonstrated beneficial
232 effects of supplementary DMG over a wide range of broiler strains, basal diets, and rearing
233 conditions. A previous tolerance and safety study demonstrated that DMG does not
234 accumulate in edible parts of broiler chickens when supplemented at a dosage of 1 g Na
235 DMG/kg and, therefore, does not pose a consumer risk of involuntary intake of DMG
236 intended as a broiler feed additive (Kalmar et al., 2012).

237

238 **Conflict of interest statement**

239 We declare that there is no conflict of interest in the publication of this paper.

240

241 **Acknowledgements**

242 Taminco NV funded the study and provided the DMG but had no role in interpretation of
243 results. There were no potential conflicts of interests.

244

245 **References**

246 AOAC, 1980. Official Methods of Analysis, 13th ed. Assoc. Off. Anal. Chem. Arlington, VA.

247 AOAC, 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem. Washington,

248 DC.

249 Craig, S.A.S., 2004. Betaine in human nutrition. *Am. J. Clin. Nutr.* 80, 539-549.

250

251 Emmert, J.L., Garrow, T.A., Baker, D.H., 1996. Hepatic betaine-homocysteine

252 methyltransferase activity in the chicken is influenced by dietary intake of sulfur amino

253 acids, choline and betaine. *J. Nutr.* 126, 2050-2058.

- 254 Freeman, C.P., 1976. Digestion and absorption of fat, in: Boorman, K.N., Freeman, B.M.
255 (Eds.), *Digestion in the fowl*. Br. Poult. Sci. Ltd, Edinburgh, pp. 117-142.
- 256 Kalmar, I.D., Cools, A., Buyse, J., Roose, P., Janssens, G.P.J., 2010. Dietary N,N-
257 dimethylglycine supplementation improves nutrient digestibility and attenuates pulmonary
258 hypertension syndrome in broilers. *J. Anim. Physiol. An. N.* 94, e339-e347.
- 259 Kalmar I.D., Cools A., Verstegen M.W. A., Huyghebaert G, Buyse J, Roose P, Janssens,
260 G.P.J., 2011. Dietary supplementation with dimethylglycine affects broiler performance
261 and plasma metabolites depending on dose and dietary fatty acid profile. *J. Anim. Physiol.*
262 *An. N.* 95, 146-153
- 263 Kalmar, I.D., Verstegen, M.W.A., Maenner, K., Zentek, J., Meulemans, G., Janssens, G.P.J.,
264 2012. Tolerance and safety evaluation of N,N-dimethylglycine (DMG), a naturally
265 occurring organic compound, as a feed additive in broiler diets. *Br. J. Nutr.* 107, 1635-
266 1644.
- 267 Klasing, K.C., 2000. *Comparative Avian Nutrition*. CAB International, Wallingford, UK.
- 268 Krogdahl, A., 1985. Digestion and absorption of lipids in poultry. *J. Nutr.* 115, 675-685.
- 269 Namroud, N.F., Shivazad, M., Zaghari, M., 2008. Effects of fortifying low crude protein diet
270 with crystalline amino acids on performance, blood ammonia level and excreta
271 characteristics of broiler chicks. *Poult. Sci.* 87, 2250-2258.
- 272 Niculescu, M.D., Craciunescu, C.N., Zeisel, S.H., 2006. Dietary choline deficiency alters
273 global and gene-specific DNA methylation in the developing hippocampus of mouse fetal
274 brains. *FASEB J.* 20, 43-49.
- 275 Prola, L., Nery, J., Lauwaerts, A., Bianchi, C., Sterpone, L., De Marco, M., Pozzo, L.,
276 Schiavone, A., 2013. Effects of N,N-dimethylglycine sodium salt on apparent
277 digestibility, vitamin E absorption, and serum proteins in broiler chickens fed a high- or
278 low-fat diet. *Poult. Sci.* 92, 1221-1226.

279 VDLUFA, 1988. Methodenbuch Band 3. VDLUFA-Verlag, Darmstadt, Germany.

280 **Table 1**
 281 Ingredient composition of basal diets (%) ¹.

Ingredient	Trial 1 (Germany)			Trial 2 (Austria)			Trial 3 (Italy)	
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter/grower	Finisher
Corn	56.49	59.26	62.21	47.24	48.03	54.29	25.20	25.20
Corn gluten meal	-	-	-	0.00	0.00	2.00	-	-
Wheat	-	-	-	10.00	10.00	10.00	27.38	30.80
Wheat DDGS ²	-	-	-	5.00	5.00	5.00	-	-
Soybean meal	33.80	29.80	26.10	29.13	26.51	18.09	27.50	20.40
Soybean extruded (whole)	-	-	-	-	-	-	10.00	12.50
Grass meal	-	-	-	1.00	2.00	3.00	-	-
Soy oil	3.70	5.00	5.70	-	-	-	5.60	6.80
Sunflower oil	1.50	1.50	1.50	-	-	-	-	-
Feed fat ³	-	-	-	3.98	4.81	4.30	-	-
Calcium carbonate/limestone	1.48	1.48	1.46	1.257	1.224	1.231	1.15	1.12
Ca(H ₂ PO ₄) ₂	1.42	1.34	1.32	1.186	1.246	1.078	1.30	1.24
Sodium bicarbonate	-	-	-	-	-	-	0.13	0.15
Sodium chloride	-	-	-	-	-	-	0.23	0.22
Vitamin-trace mineral premix ⁴	1.20	1.20	1.20	0.085	0.085	0.085	0.50	0.50
DL-Met	0.26	0.26	0.28	0.236	0.207	0.133	0.39	0.38
L-Lys.HCl	0.13	0.12	0.18	0.404	0.406	0.423	0.20	0.20
L-Thr	0.02	0.02	0.04	0.118	0.114	0.113	0.08	0.11
L-Trp	0.00	0.02	0.01	-	-	-	-	-
Choline chloride	-	-	-	0.08	0.08	0.04	0.05	0.04
Cocciostat ⁵	-	-	-	0.050	0.050	0.000	-	-
6-phytase ⁶	-	-	-	0.010	0.010	0.010	-	-
3-phytase ⁷	-	-	-	-	-	-	0.10	0.10
Antioxidant ⁸	-	-	-	0.010	0.010	0.010	-	-
1,4 beta-xylanase ⁹	-	-	-	-	-	-	0.20	0.20

282 ¹ Basal diets were divided into 2 batches, and 0 or 1 g dimethylglycine sodium salt was added per kg diet.

283 ² DDGS = dried distiller's grains and solubles (Actiprot, Wien, Austria).

284 ³ Mixture of 50% animal fat and 50% vegetable oil.

285 ⁴ Provided vitamins and trace minerals per kilogram of diet. Trial 1 (Germany): 4,800 IU vitamin A; 480 IU vitamin D₃; 50.4 mg vitamin E;
286 2.4 mg vitamin K₃; 2.4 mg vitamin B₁; 3.0 mg vitamin B₂; 42 mg niacin; 4.8 mg vitamin B₆; 0.04 mg vitamin B₁₂; 240 mg biotin; 18 mg calcium
287 pantothenate acid; 1.2 mg folic acid; 60 mg Zn; 90 mg Fe; 60 mg Mn; 14.4 mg Cu; 0.60 mg I ; 0.48 mg Co; 0.42 mg Se; 1.6 g Na; 2.0 g Mg;
288 and 1,300, 1,000, or 700 mg (starter, grower, or finisher, respectively) choline. Trial 2 (Austria): 34,000 IU vitamin A; 14,000 IU vitamin D; 0.14
289 mg vitamin E; 11.48 µg vitamin K; 8.50 µg vitamin B₁; 21.25 µg vitamin B₆; 63.75 µg vitamin B₁₂; 195.50 mg niacin; 55.25 mg pantothenic
290 acid; 5.53 µg folic acid; 0.34 µg biotin; 102 mg Fe; 102 mg Zn; 153 mg Mn; 25.5 mg Cu; 1.7 mg I; 1.7 mg Co; and 0.68 mg Se. Trial 3 (Italy;
291 starter and grower): 17,500 IU vitamin A; 30 mg vitamin E; 5 mg vitamin K₃; 3 mg vitamin B₁; 6 mg vitamin B₂; 2.5 mg vitamin B₆; 30 µg
292 vitamin B₁₂; 200 µg biotin; 20 mg Ca panthothenic acid; 750 µg folic acid; 75 mg vitamin C; 40 mg niacin; 75 mg Zn, 79 mg Fe; 71.15 mg Mn;
293 27.5 mg Cu; 925 µg I; 350 µg Co; 270 µg Se; 200 µg Mo; 125 mg DL-Met; and 125 mg BHT. Trial 3 (Italy; finisher): 12,500 IU vitamin A;
294 5,000 IU vitamin D₃; 50 mg vitamin E; 3.5 mg vitamin K₃; 2 mg vitamin B₁; 4 mg vitamin B₂; 2 mg vitamin B₆; 20 µg vitamin B₁₂; 150 µg
295 biotin; 14 mg Ca panthothenate acid; 500 µg folic acid; 75 mg vitamin C; 28 mg niacin; 52.5 mg Zn, 54.6 mg Fe; 49.75 mg Mn; 17.75 mg Cu;
296 685 µg I; 250 µg Co; 350 µg Se; 150 µg Mo; 125 mg DL-Met; and 125 mg BHT.

297 ⁵ Monensin sodium 20% (Elancoban; Elanco Animal Health, Wien, Austria).

298 ⁶ Natuphos G500 (BASF, Limburgerhof, Germany).

299 ⁷ Phytase/ZY (DSM, Basel, Switzerland).

300 ⁸ Endox (Kemin Industries Inc., Des Moines, US).

301 ⁹ Belfeed B1100MP (Beldem SA, Andenne , Belgium).

302

303

Table 2Nutrient composition (g/kg) and metabolizable energy content (MJ/kg) of basal diets (as-fed) ¹.

Item	Trial 1 (Germany) ²			Trial 2 (Austria) ³			Trial 3 (Italy) ⁴	
	Starter	Grower	Finisher	Starter	Grower	Finisher	Starter/grower	Finisher
DM	912	915	924	889	886	884	902	906
NfE	485	500	520	512	531	542	508	479
CP	222	209	187	211	189	178	205	201
EE	117	118	129	74	82	76	96	111
CA	57	57	55	56	56	51	56	76
CF	31	31	33	37	38	38	37	39
ME _n	12.6	13.1	13.3	13.0	13.0	12.9	13.4	13.8

¹ DM: dry matter, NfE: N-free extract, CP: crude protein, EE: ether extract, CA: crude ash, CF: crude fibre, and ME_n: metabolisable energy corrected at zero N-balance. Basal diets were divided into 2 batches, and 0 or 1 g dimethylglycine sodium salt was added per kg diet.

² Standard methods of VDLUFA (1988); carried out by the Institut für Tierernährung (Free University of Berlin, Berlin, Germany).

³ Standard methods of the AOAC (1980); carried out by the Futtermittel-Labor Rosenau (Wieselburg, Austria).

⁴ Standard methods of the AOAC (2000); carried out by the Dipartimento di Scienze Veterinarie (University of Torino, Torino, Italy).

304

305

306 **Table 3**
 307 Effect of dimethylglycine (DMG) as a feed additive on growth performance and carcass characteristics of broiler chickens (Trial 1) ¹.

Item	Male (n = 6)		Female (n = 6)		SEM	P-value		
	Control	DMG	Control	DMG		Diet	Sex	Diet x sex
BW (g)								
Initial	45.0	45.0	42.2	42.2	0.3	0.986	0.001	0.991
Final	2,287	2,335	2,143	2,210	23	0.130	0.001	0.787
Growth performance								
ADG (g/d)	58	59	54	56	0.6	0.130	0.002	0.787
ADFI (g/d)	89	88	82	81	0.9	0.435	0.001	0.834
FCR (g/g)								
Starter	1.42	1.25	1.40	1.32	0.01	0.004	0.562	0.265
Grower	1.45	1.44	1.50	1.48	0.01	0.479	0.037	0.813
Finisher	1.67	1.62	1.59	1.50	0.02	0.065	0.012	0.683
Overall	1.55	1.49	1.52	1.46	0.01	0.007	0.209	0.881
Mortality (%)	1.7	0.8	0.0	0.8	0.4	1.000	0.304	-
PV	373	399	360	385	5	0.015	0.166	0.934
Carcass characteristics								
CW (% BW)	80.6	81.1	81.1	81.0	0.3	0.733	0.761	0.627
Meat parts (% CW)	62.6	63.6	59.3	60.3	0.8	0.574	0.059	0.988
Breast (% CW)	24.1	23.8	21.5	23.3	0.3	0.030	0.001	0.004
Legs (% CW)	27.8	30.0	27.9	27.0	0.5	0.569	0.174	0.148
Wings (% CW)	10.7	9.8	9.9	10.0	0.2	0.400	0.597	0.289
Depot fat (% CW)	2.1	1.8	1.8	1.8	0.1	0.482	0.280	0.518
Meat/fat	24.4	31.0	28.7	28.7	1.5	0.277	0.733	0.282

308 ¹ SEM: standard error of the mean; ADG: average daily gain; ADFI: average daily feed intake; BW: bodyweight; FCR: feed conversion ratio;
 309 PV: production value; and CW: cold carcass weight. Final BW at 39 d of age.

310
 311

312 **Table 4**
 313 Growth performance and carcass characteristics of broiler chickens fed a control diet or the same diet supplemented with DMG at 1 g Na-
 314 DMG/kg feed in Trials 2 and 3 ¹.

Item	Trial 2 (n = 12)				Trial 3 (n = 11)			
	Control	DMG	SEM	<i>P</i> -value	Control	Diet	SEM	<i>P</i> -value
BW (g)								
Initial BW	42	42	0	0.979	40	40	0	0.116
Final BW	2,105	2,221	17	0.001	1,736	1,750	11	0.509
Growth performance								
ADG (g/d)	57	61	0.5	0.001	48	49	0.3	0.523
ADFI (g/d)	103	107	0.7	0.002	82	79	0.6	0.027
FCR (g/g)								
Starter	1.41	1.32	0.01	0.001	1.25	1.19	0.01	0.006
Grower	1.61	1.60	0.01	0.445	1.63	1.61	0.01	0.178
Finisher	2.30	2.30	0.02	0.396	2.20	2.12	0.03	0.036
Overall	1.81	1.78	0.01	0.211	1.70	1.63	0.01	0.006
Mortality (%)	1.7	3.3	0.9	0.355	1.1	1.70	0.6	0.631
PV	318	337	4	0.058	291	304	4	0.165
Carcass characteristics								
CW (% BW)	69.3	69.9	0.2	0.177	74.6	74.4	0.4	0.757
Meat parts (% CW)	69.2	69.1	0.3	0.824	60.7	61.3	0.2	0.068
Breast (% CW)	29.1	29.6	0.3	0.407	23.6	24.1	0.1	0.057
Legs (% CW)	28.8	28.4	0.2	0.385	27.4	27.6	0.2	0.489
Wings (% CW)	11.3	11.1	0.1	0.309	9.8	9.7	0.1	0.457
Depot fat (% BW)	2.0	2.1	0.1	0.637	1.6	1.3	0.1	0.002
Meat/fat	24.9	24.5	0.7	0.757	30.0	37.4	1.5	0.013

315 ¹ DMG: dimethylglycine; SEM: standard error of the mean; ADG: average daily gain; ADFI: average daily feed intake; BW: bodyweight;
 316 FCR: feed conversion ratio; PV: production value; and CW: cold carcass weight. Final BW at 36 d of age in Trial 2 and 35 d of age in Trial 3.
 317

318 **Table 5**
 319 Organoleptic quality of roasted breast meat in chickens fed a control diet or the same diet
 320 supplemented with dimethylglycine (DMG) at 1 g Na DMG/kg feed (Trial 2; n = 24) ¹.

Item	Control	DMG	SEM	<i>P</i> -value		
				Diet	Taster	Diet x taster
Tenderness	4.95	4.85	0.07	0.546	<0.001	0.748
Juiciness	4.56	4.38	0.07	0.277	0.032	0.703
Taste	4.69	4.53	0.07	0.387	0.031	0.756

¹Based on scores between 1 to 6 (≤ 3 : below average, and ≥ 4 : above average).

321