

Effects of *N,N*-dimethylglycine sodium salt on apparent digestibility, vitamin E absorption, and serum proteins in broiler chickens fed a high- or low-fat diet

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ABSTRACT The objective of this study was to assess the effect of supplementation with sodium salt of *N,N*-dimethylglycine (DMG-Na) on apparent digestibility (AD) in broiler chickens fed low- and high-fat diets. Twenty-eight 1-d-old broiler chickens were fed one of the dietary treatments: a low-fat diet (LF) or a high-fat diet (HF) supplemented with or without 1,000 mg/kg of DMG-Na. Body weight and feed consumption were recorded at 14 and 35 d of age. Average daily growth, daily feed intake, and feed conversion ratio were calculated. The AD of DM, organic matter (OM), CP, total fat (TF), and α -tocopheryl-acetate were assessed by 2 digestibility trials (at 18–21 and 32–35 d, respectively). Serum protein and plasma α -tocopherol concentrations were assessed at 35 d of age. Final BW, feed intake, carcass, breast, and spleen weight were higher in groups fed LF than HF diets ($P = 0.048$, $P = 0.002$, $P = 0.039$,

$P < 0.001$, $P = 0.007$, respectively). Liver weight was increased in DMG-Na-unsupplemented groups ($P = 0.011$) for both fat levels. During the first digestibility trial (18–21 d), the AD of DM ($P = 0.023$), OM ($P = 0.033$), CP ($P = 0.030$), and α -tocopheryl-acetate ($P = 0.036$) was higher in the DMG-Na-supplemented group than control. Digestibility of total fat was increased by DMG-Na supplementation in the LF groups ($P = 0.038$). A trend for improvement of digestibility was observed during the second digestibility trial (32–35 d) for DM ($P = 0.089$), OM ($P = 0.051$), and CP ($P = 0.063$) in DMG-Na groups. Total serum proteins (and relative fractions) were positively influenced by DMG-Na supplementation both in LF and HF diets ($P = 0.029$). Plasma α -tocopherol concentration was higher in groups fed LF than HF diets ($P < 0.001$).

Key words: broiler chicken, dietary fat, dimethylglycine, digestibility, vitamin E

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INTRODUCTION

Dimethylglycine (DMG) is a tertiary amino acid involved in a variety of biological processes because it is an intermediary metabolite in the cellular metabolism of choline and betaine. Friesen et al. (2007) described the role of DMG as a source of glycine for glutathione synthesis. Dimethylglycine can be metabolized in liver mitochondria, rendering both its methyl groups through transmethylation of tetrahydrofolate to free glycine (Slow et al., 2004). Being a small, water-soluble molecule that is lipophilic enough to cross cellular membranes, DMG is likely to be absorbed rapidly and completely following oral administration (Cupp and Tracy, 2003). The beneficial effects of DMG on nutri-

ent digestibility could be due to the surfactant properties of DMG esters (Clapés and Infante, 2002). Previous studies in broilers (Ross-308) reported a significant improvement of apparent digestibility of carbohydrate and protein when a control diet was supplemented with 167 mg of *N,N* dimethylglycine sodium salt (DMG-Na)/kg (Kalmar et al., 2010). Cools et al. (2010) found an improvement of crude fat, CP, and nitrogen-free extract apparent digestibility when 500 mg of DMG-Na/kg of feed was supplemented in sow diets. This beneficial effect confirms the hypothesis that DMG acts as an emulsifying agent, as recently proven by Vanhauteghem et al. (2012).

Moreover, Kalmar et al. (2011) found that plasma TBARS in broiler chickens were linearly reduced by DMG supplementation in diets containing vegetable fat. Considering that oxidative stress is a well-recognized physiological factor in the pathogenesis of pulmonary hypertension in broilers (Bottje and Wideman, 1995), the antioxidant properties of DMG-Na could at-

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tenuate the progression toward this metabolic disorder (Kalmar et al., 2010).

The main objectives of the present trial was to investigate the effect of 1,000 mg/kg of DMG-Na and 2 dietary fat levels on emulsifying properties of DMG, through measurement of apparent digestibility (**AD**) and absorption of vitamin E, as a fat-soluble nutrient, and on serum proteins in broiler chickens. In addition, growth and slaughtering performance were monitored.

MATERIALS AND METHODS

Birds, Husbandry, and Diets

The present trial was performed at the animal farm of the Faculty of Veterinary Medicine of the University of Turin (Italy). The experimental protocol was designed according to the guidelines of the Italian law for care and use of experimental animals (Ministero della Salute, 1992).

Twenty-eight 1-d-old male broiler chickens (Ross 508) were divided in 4 groups, raised in floor pens (one pen/dietary treatment) until the age of 14 d. Housing conditions were the same for all groups. Birds were fed one of the following dietary treatments: a low-fat diet (**LF**, 4.5% calculated total fat) supplemented with or without 1,000 mg/kg of DMG-Na (Taminizer D, Taminco, Ghent, Belgium) and a high fat diet content (**HF**, 9.0% calculated total fat) supplemented with or without 1,000 mg/kg of DMG-Na. All diets were supplemented with 200 UI/kg of α -tocopheryl-acetate (**α -TA**; Rovimix E-50 Adsorbate, DSM Nutritional Products Ltd., Basel, Switzerland); the added fat was soybean oil. From the age of 14 d, birds were kept individually in 2-floor cages, taking care that chicks were evenly distributed across treatment.

Diets were formulated to meet or exceed NRC (1994) requirements. Table 1 reports the composition of the diets for the growing (1–21 d) and finishing (22–35 d) period. Feed and drinking water were provided ad libitum. The lighting schedule was 23L:1D during the first 3 d, followed by 18L:6D until slaughter age. Ambient temperature was kept within the thermoneutral zone. Chicks were vaccinated at hatching against coccidiosis, Newcastle disease, and infectious bronchitis.

Growth Performances

Mortality was monitored daily during the whole experimental period. Body weight and feed consumption were recorded individually, at the age of 14 and 35 d old. Average daily gain and daily feed intake were calculated for the period 14 to 35 d.

Carcass Yield

At 35 d of age, all chickens were killed by CO₂ gasping and immediately bled. Plucked and eviscerated

carcasses were weighed. Head, neck, feet, and abdominal fat were removed to obtain carcass-for-grilling. Both carcass and carcass-for-grilling weights were also expressed as percentage of live weight (**LW**). The weight of breast, thighs, heart, liver, spleen, and abdominal fat were recorded and also expressed as a percentage of LW.

AD

The AD trials were performed using the total excreta collection method. Digestibility was evaluated from 18 to 21 and from 32 to 35 d of age. Trays were placed beneath each cage, and excreta were collected daily during the test period. Individual total fresh excreta were weighed daily, and 50% was taken after thoroughly mixing the total excreta, frozen at -20°C , and lyophilized. Individual 4-d excreta was pooled for further analysis.

Feeds and excreta were analyzed for DM, ash (AOAC International, 2004), and total fat (**TF**; Folch et al., 1957). Uric acid (**UA**) content in excreta samples was determined spectrophotometrically according to the method of Terpstra and De Hart (1974). Crude protein content of excreta was calculated as follows: CP = (total nitrogen – UA-nitrogen) \times 6.25.

The determination of α -TA content in fecal sample was performed according to the modified method described by Villaverde et al. (2008). Briefly, 100 mg of feed and excreta samples were mixed with 400 μL of hot deionized water (80°C), 1 mL of 2-propanol, 0.5 g of Na₂SO₄, and 2.5 mL of extraction solvent [85% hexane (vol/vol); 15% ethyl acetate (vol/vol); 0.05% butylhydroxytoluene (wt/vol)]. After homogenization and centrifugation, the organic layer was evaporated under vacuum, and the extract was reconstituted with methanol and injected into XTerra RP18 (250 \times 4.6 mm, 5 μm particles; Waters, Milford, MA) HPLC column preceded by an Analytical Guard Cartridge System (Phenomenex, Torrance, CA). Thirty microliters of reconstituted extract was injected into the chromatographic system. The system ran isocratically with methanol (100%) as mobile phase, at a flow rate of 1.3 mL \cdot min⁻¹, and monitored with fluorescence detection ($\lambda_{\text{ex}} = 295$ nm, $\lambda_{\text{em}} = 330$ nm). The quantification of vitamin E was based on the combined use of the retention time, and co-chromatography with a α -TA commercial standard (Sigma-Aldrich, St. Louis, MO).

Coefficients of AD of dietary nutrients were calculated as follows:

$$\text{X apparent digestibility} = \left[\frac{\text{total X ingested} - \text{total X excreted}}{\text{total X ingested}} \right] \times 100,$$

where X represents DM, organic matter (**OM**), CP, TF, and α -TA.

Table 1. Composition of the basal diets used in the experiment¹

Item	Grower period (1–21 d)		Finisher period (22–35 d)	
	LF	HF	LF	HF
Ingredient (g/kg)				
Corn meal	559.6	514.6	613.7	568.7
Soybean (seeds without hulls, meal solvent extracted)	374.0	374.0	324.0	324.0
Vegetable fat (soybean oil)	25.0	70.0	25.0	70.0
Dicalcium phosphate	13.0	13.0	12.4	12.4
Calcium carbonate	11.5	11.5	11.2	11.2
Sodium chloride	2.3	2.3	2.2	2.2
Sodium bicarbonate	1.3	1.3	1.5	1.5
DL-Methionine	3.9	3.9	2.5	2.5
L-Lysine	2.0	2.0	—	—
Threonine	0.8	0.8	1.1	1.1
Vitamin and mineral starter/grower premix ²	5.0	5.0	—	—
Vitamin and mineral finisher premix ³	—	—	5.0	5.0
Choline chloride	0.4	0.4	0.2	0.2
3-phytase (E-300; natuphos bio/G500)	1.0	1.0	1.0	1.0
Vitamin E (α -tocopheryl-acetate) ⁴ (IU/kg)	200	200	200	200
Analyzed composition (g/kg)				
DM	909.7	921.2	889.8	899.6
CP	227.2	227.8	194.8	189.6
Total fat	55.9	85.3	58.1	94.5
Ash	64.1	65.4	55.8	54.3
Vitamin E (α -tocopheryl-acetate; IU/kg)	173	174	184	185
ME ⁵ (MJ/kg)	12.36	13.41	12.58	13.63
Fatty acid composition ⁶ (g/100 g of feed)				
C16:0	0.51	0.92	0.52	0.93
C16:1	0.26	0.74	0.26	0.74
C18:0	0.16	0.33	0.16	0.34
C18:1	1.17	2.12	1.21	2.16
C18:2	2.69	5.04	2.76	5.11
C18:3	0.24	0.57	0.24	0.56

¹LF = low-fat diet; HF = high-fat diet.

²Starter/grower premix (B28 Prisma, IZA SRL, Forlì, Italy) kg⁻¹: 3,500,000 IU of vitamin A; 6,000 IU of vitamin E; 1,000 mg of vitamin K₃; 600 mg of vitamin B₁; 1,200 mg of vitamin B₂; 500 mg of vitamin B₆; 6 mg of vitamin B₁₂; 40 mg of biotin; 4,000 mg of Ca pantothenate acid; 150 mg of folic acid; 15,000 mg of vitamin C; 8,000 mg of vitamin B₃; 15,000 mg of Zn; 15,800 mg of Fe; 14,230 mg of Mn; 5,500 mg of Cu; 185 mg of I; 70 mg of Co; 54 mg of Se; 40 mg of Mo; 25,000 mg of DL-methionine; 25,000 mg of butylated hydroxytoluene.

³Finisher premix (Final B Prisma, IZA SRL) kg⁻¹: 2,500,000 IU of vitamin A; 1,000,000 IU of vitamin D₃; 10,000 IU of vitamin E; 700 mg of vitamin K₃; 400 mg of vitamin B₁; 800 mg of vitamin B₂; 400 mg of vitamin B₆; 4 mg of vitamin B₁₂; 30 mg of biotin; 2,800 mg of Ca pantothenate acid; 100 mg of folic acid; 15,000 mg of vitamin C; 5,600 mg of vitamin B₃; 10,500 mg of Zn; 10,920 mg of Fe; 9,950 mg of Mn; 3,550 mg of Cu; 137 mg of I; 50 mg of Co; 70 mg of Se; 30 mg of Mo; 25,000 mg of DL-methionine; 25,000 mg of butylated hydroxytoluene.

⁴Rovimix E-50 Adsorbate, F. Hoffman-La Roche Ltd., Basel, Switzerland.

⁵Based on NRC (1994) ingredient composition.

⁶Based on FEDNA (2010) ingredient composition (<http://fundacionfedna.org/>).

Blood Protein Profile and α -Tocopherol Concentration

At the end of the experiment (d 35), blood samples were collected from the femoral vein: 2.5 mL was placed in EDTA tubes and 2.5 mL in serum-separating tubes. They were centrifuged for 15 min at 3,000 $\times g$. The plasma and serum samples were stored at -80°C pending analysis. On serum sample, total proteins were quantified using the biuret method (Bio Group Medical System kit, Hospitex Diagnostics, Sesto Fiorentino, Firenze, Italy); the serum electrophoretic patterns (albumin, α -globulin, β -globulin, and γ -globulin) were obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys, Hydragel 30 Protein, Sebia, Evry, France). Plasma samples were submitted to a private veterinary laboratory (Vet Med Labor GmbH,

Ludwigsburg, Germany) to determine α -tocopherol (α -TOC) content.

Statistical Analysis

Normality of data distribution was assessed using the Shapiro-Wilk test. Data were then analyzed with 2-way ANOVA with fat level (LF vs. HF) and DMG-Na supplementation (control vs. DMG-Na) as main factors. Differential analysis of the effect of each factor was considered whenever fat level and DMG-Na interaction was observed. The results are presented as mean values and pooled SEM. The results were considered statistically significant when associated with a probability lower than 5%. The results with a probability lower than 1% were considered highly significant. A statistical trend was considered for *P*-values below 10%.

Table 2. Growth performance and carcass yields of broiler chickens (n = 7)¹

Item	LF (4.5% dietary fat)		HF (9.0% dietary fat)		SEM	<i>P</i> -value main effect		
	Control	DMG-Na	Control	DMG-Na		Fat	DMG-Na	Fat × DMG-Na
Final BW (g; d 35)	1,574.6	1,638.6	1,502.4	1,529.4	22.743	0.048	0.305	0.674
Feed intake (g/d; 14–35 d)	106.8	106.7	97.2	98.0	1.516	0.002	0.886	0.857
Daily growth (g/d; 14–35 d)	60.2	62.9	57.0	57.8	1.048	0.051	0.398	0.660
Feed conversion ratio (14–35 d)	1.78	1.71	1.70	1.70	0.019	0.316	0.371	0.354
Chilled carcass ² (g)	1,158.7	1,227.4	1,097.1	1,112.8	21.379	0.039	0.306	0.517
Chilled carcass (% of LW)	73.4	74.8	73.0	72.8	0.523	0.242	0.577	0.464
Carcass for grilling ³ (g)	964.3	1,031.4	900.3	934.3	18.749	0.028	0.156	0.636
Carcass for grilling (% of LW)	61.2	62.9	59.8	61.1	0.586	0.202	0.207	0.854
Breast (g)	215.3	230.6	181.7	190.0	5.500	0.000	0.180	0.687
Breast (% of LW)	13.7	14.1	12.1	12.4	0.258	0.001	0.361	0.972
Thighs (g)	313.7	339.7	285.4	292.0	7.464	0.009	0.230	0.470
Thighs (% of LW)	19.9	20.7	18.9	19.1	0.300	0.039	0.386	0.574
Heart (g)	12.0	10.8	9.8	10.2	0.440	0.126	0.645	0.364
Heart (% of LW)	0.7	0.6	0.6	0.7	0.023	0.304	0.346	0.231
Liver (g)	36.4	26.6	34.6	29.4	1.474	0.856	0.011	0.412
Liver (% of LW)	2.3	1.6	2.3	1.9	0.101	0.343	0.003	0.368
Spleen (g)	2.6	2.7	2.0	2.1	0.109	0.007	0.698	0.789
Spleen (% of LW)	0.2	0.2	0.1	0.1	0.007	0.035	0.927	0.857
Abdominal fat (g)	21.4	22.6	26.6	26.5	0.757	0.002	0.660	0.584
Abdominal fat (% of LW)	1.3	1.4	1.8	1.7	0.046	0.000	0.825	0.526

¹LW = live weight; LF = low-fat diet; HF = high-fat diet; DMG-Na = *N,N* dimethylglycine sodium salt.

²Carcass: plucked and eviscerated carcasses after chilling at 4°C.

³Carcass for grilling: carcass without head, neck, feet, and abdominal fat.

RESULTS

No mortality was observed during the trial. Table 2 reports growth performance of broiler chickens. Final BW was influenced by dietary fat level ($P = 0.048$), with higher BW for LF than HF groups. Feed intake was higher in LF than in HF groups ($P = 0.002$) irrespective of DMG-Na supplementation. The same trend was observed for daily growth ($P = 0.051$). The feed conversion ratio (14–35 d) was neither influenced by dietary fat level nor by DMG-Na supplementation.

Carcass yields were mainly influenced by dietary fat content. The weight of chilled carcass, carcass for grilling, breast, and spleen were higher in groups fed LF than HF diets ($P = 0.039$, $P = 0.028$, $P < 0.001$, and $P = 0.007$, respectively). Abdominal fat weight was higher in groups fed HF than LF diets ($P = 0.002$). Liver weight was increased in DMG-Na-unsupplemented groups ($P = 0.011$) for both fat levels (Table 2).

The AD of DM, OM, and CP during the first digestibility trial (growing phase, 18–21 d, Table 3) were positively influenced by both dietary fat level ($P = 0.025$, $P = 0.021$, and $P = 0.002$, respectively) and DMG-Na supplementation ($P = 0.023$, $P = 0.033$, and $P = 0.030$, respectively). An interaction between fat level and DMG-Na supplementation was found for TF digestibility ($P = 0.029$). Digestibility of TF was positively influenced in DMG-Na-supplemented LF groups, whereas no effect was found in DMG-Na-supplemented HF groups. Digestibility of α -TA was influenced by DMG-Na supplementation ($P = 0.036$) irrespective of dietary fat level.

The AD of DM, OM, and TF was influenced by dietary fat content ($P = 0.010$, $P = 0.006$, and $P < 0.001$ respectively), and a statistical trend was observed for CP ($P = 0.078$) during the second digestibility trial (finishing phase, 32–35 d, Table 3). A statistical trend improvement for digestibility was observed in DMG-Na supplemented groups for DM ($P = 0.089$), OM ($P = 0.051$), and CP ($P = 0.063$). Digestibility of α -TA was not affected by dietary treatments.

Blood concentration of protein fractions and α -TOC are presented in Table 4. Total serum protein (and relative fractions) was positively influenced by DMG-Na supplementation. Plasma α -TOC concentration was higher in groups fed LF than groups fed HF diets ($P < 0.001$), irrespective of DMG-Na supplementation.

DISCUSSION

Beneficial effects of DMG-Na supplementation in broiler chicken were previously reported in the literature. In particular, previous studies suggested that DMG-Na supplementation would lead to improvement of performance traits (Kalmar, 2011), decreased plasma TBARS (Kalmar et al., 2011), and increased nutrient digestibility (Kalmar et al., 2010). In this context, the present study was conducted to assess the effect of DMG-Na dietary supplementation on apparent digestibility in broiler chickens fed 2 levels of dietary fat.

Final BW and feed intake were higher for chickens fed LF than HF diets. No effects of DMG-Na supplementation on performance traits were observed. However, the rearing conditions used in the present study (i.e., indi-

Table 3. Nutrient digestibility (%) of broiler chicken at the age of 18 to 21 d and 32 to 35 d (n = 7)¹

Item	LF (4.5% dietary fat)		HF (9.0% dietary fat)		SEM	<i>P</i> -value main effect		
	Control	DMG-Na	Control	DMG-Na		Fat	DMG-Na	Fat × DMG-Na
First digestibility trial (growing phase, 18–21 d)								
dDM ² (%)	73.6	75.0	75.0	78.0	0.532	0.025	0.023	0.415
dOM ³ (%)	74.7	75.9	76.1	78.9	0.508	0.021	0.033	0.381
dCP ⁴ (%)	79.3	81.6	82.9	85.0	0.610	0.002	0.030	0.998
dTF ⁵ (%)	78.0 ^a	81.4 ^b	88.1	88.0	0.909	0.000	0.038	0.029
dα-TA ⁶ (%)	67.8	74.5	67.8	72.1	1.458	0.595	0.036	0.593
Second digestibility trial (finishing phase, 32–35 d)								
dDM (%)	71.7	72.6	73.1	74.2	0.305	0.010	0.089	0.795
dOM (%)	73.8	74.7	75.1	76.2	0.288	0.006	0.051	0.861
dCP (%)	77.1	77.8	77.7	81.2	0.594	0.078	0.063	0.215
dTF (%)	80.4	81.4	87.7	89.6	0.895	0.000	0.151	0.629
dα-TA (%)	62.2	62.2	58.8	60.0	1.545	0.378	0.876	0.855

^{a,b}*P* < 0.05.

¹LF = low-fat diet; HF = high-fat diet; DMG-Na = *N,N* dimethylglycine sodium salt.

²dDM: digestibility of DM.

³dOM: digestibility of organic matter.

⁴dCP: digestibility of CP.

⁵dTF: digestibility of total fat.

⁶dα-TA: digestibility of α-tocopheryl acetate.

vidual housing in cages) did not reflect the commercial rearing practice. This would possibly explain the lack of the significant effect of DMG-Na supplementation on feed conversion ratio as reported in other studies (Kalmar, 2011; Ortega Sánchez de Tagle et al., 2011).

Liver weight was reduced in DMG-Na-supplemented groups compared with controls. This result could be related to a better hepatic function due to the interaction between DMG and methylation reaction occurring in the liver. The DMG is a naturally occurring intermediary metabolite in the choline to glycine metabolism, and acts as a methyl donor (Tonda and Hart, 1992; Slow et al., 2004). Kalmar et al. (2011) suggested that in poultry DMG may play a sparing effect on choline, which can be used for its specific functions other than methylation. Specifically, choline is considered a lipotropic factor due to its function in the regulation of hepatic lipoprotein synthesis. Furthermore DMG is a precursor of glycine synthesis. During early life, glycine is considered as an essential amino acid in chickens due to insufficient biosynthesis rate (Klasing, 2000). There-

fore, DMG supplementation could also lead to improvement of protein biosynthesis in birds during early life.

The higher plasma α-TOC observed in chickens fed LF compared with HF diets could be a consequence of reduced polyunsaturated fatty acid (PUFA) ingestion in birds fed LF diets, and a consequent sparing effect on vitamin E. It is well known that the dietary requirement of vitamin E increases linearly with the amount of PUFA in the diet (Klasing, 2000). Nevertheless, there are currently controversial arguments on the interaction of vitamin E absorption and dietary fat level in broiler chickens (Villaverde et al., 2008). Previous authors suggested that in other species, increased dietary PUFA led to decreased bioavailability of vitamin E following degradation in the intestinal tract (Tijburg et al., 1997). This could explain the higher plasma α-TOC concentrations in chicken fed LF compared with HF diets found in the present study.

A significant positive effect of DMG-Na supplementation on AD was observed during the growing phase. The DMG-Na supplementation improved the AD of

Table 4. Serum proteins and plasma α-tocopherol of broiler chicken at the age of 35 d (n = 7)¹

Item	LF (4.5% dietary fat)		HF (9.0% dietary fat)		SEM	<i>P</i> -value main effect		
	Control	DMG-Na	Control	DMG-Na		Fat	DMG-Na	Fat × DMG-Na
Total protein (g/dL)	1.69	2.10	1.94	2.41	0.103	0.147	0.029	0.860
Albumin (g/dL)	0.70	0.82	0.80	0.94	0.033	0.077	0.039	0.841
α-Globulin (g/dL)	0.48	0.58	0.55	0.66	0.026	0.128	0.035	0.892
β-Globulin (g/dL)	0.25	0.38	0.31	0.36	0.019	0.648	0.026	0.330
Gamma globulin (g/dL)	0.23	0.32	0.29	0.43	0.028	0.118	0.035	0.562
Albumin/globulin ratio	0.73	0.65	0.70	0.67	0.011	0.813	0.014	0.404
Plasma α-tocopherol (mg/L)	40.0	42.3	29.9	32.7	1.277	0.000	0.154	0.892

¹LF = low-fat diet; HF = high-fat diet; DMG-Na = *N,N* dimethylglycine sodium salt.

DM, organic matter, and CP in LF and HF diets. Fat digestibility was also improved by DMG-Na supplementation in LF diets. Dietary supplementation of DMG-Na also improved digestibility of α -TA during the growing phase, probably as a consequence of the emulsifying effect of DMG-Na on the lipid ingesta fraction (Kalmar et al., 2010). During the finishing phase, a positive trend was observed for AD of DM, organic matter, and CP.

Serum proteins were higher in birds fed DMG-Na-supplemented diets than birds fed control diets. Serum albumin concentration depends on hepatic synthesis rate, degradation, release from liver, body distribution, and exogenous loss (Lumeij, 2008; Thalacker-Mercer and Campbell, 2008). In our experiment, the highest serum albumin and total protein observed in broilers fed DMG-Na-supplemented diets could therefore be related to improved protein digestibility compared with nonsupplemented groups and to increased availability of amino acids precursors for protein synthesis. This could lead to an optimization of the oncotic pressure and, consequently, ascites prevention (Currie, 1999).

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REFERENCES

- AOAC International. 2004. Official Methods of Analysis. 18th ed. AOAC Int., Arlington, VA.
- Bottje, W. G., and R. F. Wideman. 1995. Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome. *Poult. Avian Biol. Rev.* 6:211–231.
- Clapés, P., and M. R. Infante. 2002. Amino acid-based surfactants: Enzymatic synthesis, properties and potential applications. *Biotransform.* 20:215–233.
- Cools, A., D. Maes, J. Buyse, I. D. Kalmar, J. A. Vandermeiren, and G. P. J. Janssens. 2010. Effect of *N,N*-dimethylglycine supplementation in parturition feed for sows on metabolism, nutrient digestibility and reproductive performance. *Animal* 4:2004–2011.
- Cupp, M. J., and T. S. Tracy, ed. 2003. Dimethylglycine (*N,N*-dimethylglycine). Pages 149–160 in *Dietary Supplements: Toxicology and Clinical Pharmacology*. Humana Press, Totowa, NJ.
- Currie, R. J. W. 1999. Ascites in poultry: Recent investigations. *Avian Pathol.* 28:313–326.
- Folch, J., M. Lees, and H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* 226:479–509.
- Friesen, R. W., E. M. Novak, D. Hasman, and S. M. Innis. 2007. Relationship of dimethylglycine, choline, and betaine with oxoprolone in plasma of pregnant women and their newborn infants. *J. Nutr.* 137:2641–2646.
- FEDNA (Fundación Española para el Desarrollo de la Nutrición Animal). 2010. Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos. <http://fundacionfedna.org/>.
- Kalmar, I. D. 2011. Efficacy and safety of dietary *N,N*-dimethylglycine in broiler production. PhD Thesis. Wageningen University, Wageningen, the Netherlands.
- Kalmar, I. D., A. Cools, J. Buyse, P. Roose, and G. P. J. Janssens. 2010. Dietary *N,N*-dimethylglycine supplementation improves nutrient digestibility and attenuates pulmonary hypertension syndrome in broilers. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 94:e339–e347.
- Kalmar, I. D., A. Cools, M. W. A. Verstegen, G. Huyghebaert, J. Buyse, P. Roose, and G. P. J. Janssens. 2011. Dietary supplementation with dimethylglycine affects broiler performance and plasma metabolites depending on dose and dietary fatty acid profile. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 95:146–153.
- Klasing, K. C. 2000. *Comparative Avian Nutrition*. CAB International, Wallingford, UK.
- Lumeij, J. T. 2008. Avian clinical biochemistry. Pages 839–872 in *Clinical Biochemistry of Domestic Animals*. J. J. Kaneko, J. W. Harvey, and M. L. Bruss, ed. Elsevier Academic Press, Oxford, UK.
- Ministero della Salute. 1992. Decreto Legislativo del 27 Gennaio 1992, n. 116. *Gazzetta Ufficiale della Repubblica Italiana*, n. 40.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. Natl. Acad. Press, Washington, DC.
- Ortega Sánchez de Tagle, J., A. Ortiz Muñiz, J. C. del Río, M. A. Carmona Medero, and A. Lauwaerts. 2011. Resultados experimentales con el uso del *N,N*-dimetilglicinato de sodio (Na-DMG) Taminizer-D® en pollo de engorda en Mexico. Pages 881–887 in *Proceedings of XVII WVPA Congress*, 14–18 August, Cancun, Mexico.
- Slow, S., D. O. McGregor, M. Lever, M. B. Lee, P. M. George, and S. T. Chambers. 2004. Dimethylglycine supplementation does not affect plasma homocysteine concentrations in pre-dialysis chronic renal failure patients. *Clin. Biochem.* 37:974–976.
- Terpstra, K., and N. De Hart. 1974. The estimation of urinary nitrogen and faecal nitrogen in poultry excreta. *Z. Tierphysiol. Tierernahr. Futtermittelkd.* 32:306–320.
- Thalacker-Mercer, A. E., and W. W. Campbell. 2008. Dietary protein intake affects albumin fractional synthesis rate in younger and older adults equally. *Nutr. Rev.* 66:91–95.
- Tijburg, L. B., E. Haddeman, G. A. Kivits, J. A. Weststrate, and E. J. Brink. 1997. Dietary linoleic acid at high and reduced dietary fat level decreases the faecal excretion of vitamin E in young rats. *Br. J. Nutr.* 77:327–336.
- Tonda, M. E., and L. L. Hart. 1992. *N,N* dimethylglycine and L-carnitine as performance enhancers in athletes. *Ann. Pharmacother.* 26:935–937.
- Vanhautehem, D., G. P. J. Janssens, A. Lauwaerts, S. Sys, F. Boyen, I. D. Kalmar, and E. Meyer. 2012. Glycine and its *N*-methylated analogues cause pH-dependent membrane damage to enterotoxigenic *Escherichia coli*. *Amino Acids* 43:245–253.
- Villaverde, C., M. D. Baucells, E. G. Manzanilla, and A. C. Barroeta. 2008. High levels of dietary unsaturated fat decrease α -tocopherol content of whole body, liver, and plasma of chickens without variations in intestinal apparent absorption. *Poult. Sci.* 87:497–505.