Dietary Methionine Intake Effects on Egg Component Yield, Composition, Functionality, and Texture Profile Analysis

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ABSTRACT The influence of supplemental Met levels ranging from 413 to 556 mg per hen per d (mg/HD) on liquid egg component yield, composition, and functionality was examined in mature layers (29 wk of age). Egg weight, component yield, solids, and CP content of albumen and yolk were determined. Texture profile analysis, feed ingredient functionality testing, and PAGE were conducted to determine whether increased egg total solids and CP content resulted in altered egg component functionality or electrophoretic protein banding pattern.

Albumen component yield increased significantly on a mass basis at 507 and 556 mg/HD Met compared to 413 mg/HD Met. Yolk mass yield was significantly increased at 556 mg/HD Met compared to 413 mg/HD Met. Consumption above 413 mg/HD Met resulted in significantly increased albumen total solids and protein. Yolk solids were not significantly different; however, yolk CP was significantly increased at 507 and 556 mg/HD Met compared to 413 mg/HD Met. Albumen and yolk functionality at 413 and 507 g/HD Met were not significantly different in relation to cake volume or height. Emulsion separation at 120 min was significantly increased for 556 mg/HD Met compared to 413 and 507 g/HD Met. There were no significant differences in hardness or springiness of albumen and yolk gel plugs and electrophoretic protein banding patterns. Increased understanding of the influence of Met on liquid egg yield and composition may provide the egg producer with an effective and advantageous management technique for shell egg production specifically managed to maximize liquid egg product.

(Key words: methionine, egg component, protein, solids, functionality)

INTRODUCTION

Increasing numbers of liquid egg (LE) processing plants has led to dedication of layer flock production to meet processor needs. Research into nutritional effects on LE yield and composition may provide the egg producer an effective management tool for customized shell egg production (EP) specifically managed to maximize the yield of liquid egg product (LEP). The LEP are produced by mechanical separation of albumen and yolk from the shell and marketed in liquid or frozen form prior to sale through commercial and retail markets. Liquid egg product is pasteurized and sold as either whole egg, albumen, yolk, or a specified blend. As per capita shell egg consumption continues to decrease, LEP represents the segment of the commercial egg industry exhibiting growth. From 1980 to 1996, U.S. LE consumption has steadily increased to 62.1 LE per capita (USDA, 1997). Consumption of LE has expanded through retail consumer products and LEP utilization in hotel, restaurant, and institutional services.

Standard parameters by which LEP are evaluated and marketed include percentage yolk and albumen solids. Percentage solids, commonly referred to as total or dried solids, is the nonaqueous component remaining after water removal. In LEP, minimum solids content may be specified by a processor, customer, or regulatory agency. A second parameter of major importance to LE production is component yield including, albumen, yolk, and shell. Albumen and yolk are the valued products, whereas shell is treated as a low value by-product, or wastage, by breaking operations. Increasing proportional liquid component yield will allow processors to produce greater liquid mass from an equivalent number of eggs.

Methionine, an essential dietary amino acid, is used to synthesize proteins and other amino acids. As a
limiting nutrient in commercially prepared corn and soybean meal layer rations, DL-Met crystalline forms or Met hydroxy analogs (MHA) are commonly used as supplements. The MHA supported EP and egg size equivalent to that for Met when diets were formulated at or above NRC recommendations (Damron and Harms, 1972). No significant differences were reported in the effectiveness of DL-Met vs MHA when fed in equimolar amounts to layers (Reid et al., 1982).

Carey and coworkers (1991) increased egg component weight by increasing Met intake levels from 326 to 512 mg per hen per d (mg/HD). Significantly increased egg weight and component mass were produced by hens at the higher supplementation level. Albumen and yolk solids increased significantly \( (P < 0.0001) \), on a mass basis, in eggs produced by layers fed 512 mg/HD Met.

Additionally, Shafer et al. (1996) reported increases in albumen and yolk protein at Met intakes of 392 and 423 mg/HD compared to 328 and 354 mg/HD. In a similar amino acid study, increased Lys intake by layers, fed sorghum and soybean meal diets supplemented with Lys, resulted in significant increases in albumen and yolk weight and protein and albumen solids (Prochaska et al., 1996).

Further experiments were conducted to evaluate the influence of increased Met intake on LE component yield, solids, and CP content. Additionally, relational influences on food functionality and electrophoretic banding patterns were examined.

**MATERIALS AND METHODS**

The experimental design consisted of three dietary treatments assigned randomly within each of three replications. Experimental units consisted of nine laying hens, with three experimental units in each replication group. Layers were individually caged in the lower level of a two-deck system with cage dimensions of 25.4 × 45.7 cm. A total of 81 mature layers of a commercial strain were weighed and assigned to experimental units with calculated Met concentrations of 0.38, 0.46, and 0.53% within 4 h. Eggs were analyzed for component yield by increasing Met intake levels from 326 to 512 mg per hen per d (mg/HD). Significantly increased egg weight and component mass were produced by hens at the higher supplementation level. Albumen and yolk solids increased significantly \( (P < 0.0001) \), on a mass basis, in eggs produced by layers fed 512 mg/HD Met.

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Further experiments were conducted to evaluate the influence of increased Met intake on LE component yield, solids, and CP content. Additionally, relational influences on food functionality and electrophoretic banding patterns were examined.

**Diet Formulation and Feeding**

Diet Formulation and Feeding

Diets were formulated such that 105 g/HD feed intake would meet the NRC recommendations (NRC, 1994) for all nutrients (Table 1). Crude protein was formulated at 16.5% for all diets (Table 1). A corn and soybean meal basal ration was prepared at 21-d intervals and divided into nine aliquots weighing 22.5 kg. These aliquots of feed were mixed with a premix packet (227 g) containing MHA and soybean meal using a Hobart mixer. The premix contained all supplemental MHA in the ration and sufficient soybean meal to reach a total premix weight of 227 g. This procedure resulted in complete layer rations with calculated Met concentrations of 0.38, 0.46, and 0.53% (Table 1). Feed samples were collected from each mixing date, blended, pooled by treatment, and submitted for proximate analysis and Met content. Feed consumption was determined on a weekly basis.

**Egg Component Analysis**

Egg production and egg weight were calculated on a weekly basis. On each predetermined sample day, all eggs from each experimental unit were collected during a 24-h period, weighed, and analyzed for component yield within 4 h. Eggs were analyzed for component yield by

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3. Monodicalcium phosphate source.
4. Provides per kilogram of diet: retinyl acetate, 9,900 IU; cholecalciferol, 2,750 IU; dl-α-tocopherol acetate, 27.5 IU; menadione, 2.2 mg; thiamine mononitrate, 2.16 mg; riboflavin, 6.6 mg; vitamin B12, 0.022 mg; niacin, 44 mg; choline, 495 mg; d-pantothenic acid, 11.0 mg; pyridoxine, 3.96 mg; biotin, 0.11 mg; folic acid, 1.1 mg; ethoxyquin, 55 mg.
5. Provides per kilogram of diet: manganese oxide, 68.2 mg; zinc oxide, 55 mg; ferrous sulfate, 26.4 mg; copper sulfate, 4.4 mg; calcium iodate, 1.1 mg; sodium selenite, 0.1 mg.
mass for yolk, albumen, and shell with membranes intact. Yolk and albumen were separated manually using a plastic egg separator and rubber spatula. Excess albumen was removed from the yolk by blotting with damp paper towels. Weight of yolk and wet shell with shell membranes intact were subtracted from whole egg weight to calculate albumen weight for each individual egg as described by Fletcher et al. (1981). Separated components were pooled by experimental units, stored in resealable polyethylene food storage bags5 (17.7 cm × 13.3 cm) and refrigerated at 5°C for further analyses the following day.

**Component Solids and Protein Determination**

Upon removal from refrigeration, samples were homogenized with a hand-held blender6 using a 20-s pulse. Component solids and CP were measured from three 10-g aliquots from each albumen and yolk pooled sample described previously. Yolk and albumen homogenate from each experimental unit were individually pipetted into tared aluminum drying pans and weights were recorded to 0.0001 g. Aliquots were dried in a convection oven for 24 h at 105°C (AOAC, 1984), removed from the oven, allowed to cool in a desiccator, and weighed within 30 min. Kjeldahl digestion and distillations were performed using two aliquots of albumen and yolk per experimental unit analyzed. Crude protein was calculated from total nitrogen determination by standard Kjeldahl procedures for nitrate-free samples (AOAC, 1984). Nitrogen determinations, obtained by titration, were corrected against two standardized blanks accompanying each digestion of 18 tubes. Protein values were calculated by multiplying nitrogen content by a factor of 6.25.

**Functional Performance and Texture Profile Analysis**

Comparison of egg components from the dietary treatments were examined for functional performance in baked angel or sponge cakes and yolk emulsions. Angel and sponge cakes were prepared using standardized recipes and methodology for ingredients and procedures (Froning et al., 1986). Angel cake was used to examine functional baking qualities of albumen and sponge cake was used for yolk evaluation. Cakes were prepared from eggs collected the day prior to preparation and allowed to equilibrate to room temperature for 2 h. One sample date was utilized for each type of cake preparation. Yolks were strained through two layers of cheesecloth to remove yolk membranes prior to use in sponge cake preparation.

Batter was prepared for all cakes using a multispeed blender and stainless steel mixing bowls. Batter was placed in nonstick aluminum cake pans measuring 14×8.3×4.7 cm with a total volume of 546 cm³. Batter was dispensed in 65-g aliquots per pan for angel cake and 84 g per pan for sponge cake. Angel cakes and sponge cakes were baked in conventional gas fired ovens on consecutive days. Two cakes were prepared from each experimental unit sample and baked in the same randomly assigned oven. Angel and sponge cakes were baked for 20 and 25 min at 190°C, respectively. Cake height and volume were recorded by rapeseed displacement.

Yolk emulsion separation was determined according to the method described by Varadarajulu and Cunningham (1972). Emulsions were prepared using 15 g of a commercially available corn oil, 15 g of egg yolk homogenate, and 85 mL of distilled water. Each mixture was homogenized in a 250-mL glass homogenizer cup using a Virtis, model 23, homogenizer7 for 2 min at high speed (23,000 rpm). Blade height was maintained at 6 mm from the bottom of the homogenizer cup during emulsion homogenization. Each emulsion preparation was immediately divided into five portions by pipetting into tapered, graduated 15-mL polycarbonate centrifuge tubes. Tubes were placed upright in test tube racks, time documented, and volume of aqueous separation per tube was read at 60 and 120 min elapsed time. Emulsion preparation and evaluation was repeated over four sample dates for a total of 240 samples.

Texture profile analysis (TPA) was conducted on heat-formed gels of albumen and yolk using the method described by Woodward and Cotterill, (1987) with a modification of the gel plug preparation and size. Albumen samples were blended using a hand blender6 for a 20-s pulse. Yolk was stirred with a glass rod then strained through two layers of cheesecloth. No water or pH adjustments were made to the yolk samples. Aliquots of 25 mL per component sample were dispensed into 30-mL beakers lightly coated with nonstick spray and over-wrapped with aluminum foil. Samples were placed in a water bath in which the water depth was 1 cm below the lip of the beaker. Albumen and yolk samples were heated for 35 and 30 min at 80°C, respectively. Samples were removed from the water bath and allowed to cool to room temperature then refrigerated at 5°C until the next day. Samples were removed from refrigeration and allowed to equilibrate to room temperature. Gelatinized samples were removed from the beakers with a thin stainless steel spatula and cylinders were trimmed to a length of 2.5 cm using a modified egg slicer.

The TPA was performed using an Instron Universal Testing Machine8 (Model 1011) equipped with a compression anvil and plate. Two samples per experimental unit were analyzed over four dates for a total of 144 samples each for yolk and albumen. The TPA was conducted using a 500 N load cell. Albumen gel plug samples were subjected to a 50 N load range and yolk gel plugs were subjected to a 100 N load range. Gel plugs were subjected

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5 Dowbrands Inc., Indianapolis, IN 46268-0511.
6 Braun Inc., Lynnfield, MA 01940.
7 Virtis Research Equipment, Gardiner, NY 12525.
8 Instron Corp., Canton, MA 02021.
**TABLE 2. Feed consumption, calculated intake of methionine, crude protein, metabolizable energy, lysine, and total sulfur amino acid**

<table>
<thead>
<tr>
<th>Methionine level (%)</th>
<th>Feed consumption (g/HD)</th>
<th>Methionine (mg/HD)</th>
<th>Crude protein (g/HD)</th>
<th>ME (kcal/HD)</th>
<th>Lysine (mg/HD)</th>
<th>TSAA (mg/HD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.38%</td>
<td>108&lt;sup&gt;A&lt;/sup&gt;</td>
<td>413&lt;sup&gt;C&lt;/sup&gt;</td>
<td>17.9&lt;sup&gt;A&lt;/sup&gt;</td>
<td>304&lt;sup&gt;A&lt;/sup&gt;</td>
<td>947&lt;sup&gt;A&lt;/sup&gt;</td>
<td>718&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.46%</td>
<td>110&lt;sup&gt;A&lt;/sup&gt;</td>
<td>507&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>308&lt;sup&gt;A&lt;/sup&gt;</td>
<td>959&lt;sup&gt;A&lt;/sup&gt;</td>
<td>815&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.53%</td>
<td>105&lt;sup&gt;B&lt;/sup&gt;</td>
<td>556&lt;sup&gt;A&lt;/sup&gt;</td>
<td>17.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>293&lt;sup&gt;B&lt;/sup&gt;</td>
<td>912&lt;sup&gt;B&lt;/sup&gt;</td>
<td>849&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>n</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.51</td>
<td>2.26</td>
<td>0.08</td>
<td>1.44</td>
<td>4.48</td>
<td>3.69</td>
</tr>
</tbody>
</table>

<sup>A</sup>-<sup>C</sup> Means within a column with no common superscript differ significantly (<i>P</i> < 0.01).

<sup>1</sup>Calculated from feed consumption data.

<sup>2</sup>HD = per live hen per day.

Native yolk proteins were examined using non-denaturing PAGE (ND-PAGE) reagents in kit form.<sup>9</sup> Yolk samples were prepared by modification of methods reported by Chang <i>et al.</i> (1970) and Dixon and Cotterill (1981). Native egg yolk was stirred with a glass rod till homogeneous, then passed through two layers of cheesecloth. Each sample was prepared by dilution of 2 mL egg yolk to 100 mL of ND sample buffer (0.0625 M Tris-HCl (pH 6.8), 15% glycerol, and 0.002% bromphenol blue dye). The diluted sample was loaded by volume, 5 μL per lane, onto a 4.0% T stacking gel with 9.5% T resolving gel. The ratio of acrylamide to N, N<sub>1</sub>-methylene bis-acrylamide was 29.2:0.8. Molecular weight markers for ND-PAGE were obtained and loaded according to manufacturer’s instructions.<sup>9</sup> Gels were run and destained using the same methodology as for SDS-PAGE. Electrophoretic patterns of the protein bands were scanned for optical density by computer imaging using Visage 1-D analysis software<sup>10</sup> for single lane analysis and multiple lane comparisons.

**Statistical Analysis**

All data were subjected to ANOVA utilizing the General Linear Models procedure of SAS® (1990) statistical analysis software program, Version 6.04, with the main effects being diet, date, and replication. Mean differences were separated via the PDIVF option, which uses pairwise <i>t</i> tests, of the General Linear Model option. Least squares means and SE were determined.<sup>7</sup>

**RESULTS**

Based on feed consumption data and analysis of feed samples, the Met intake of hens fed the 0.38, 0.46, and 0.53% Met treatments were calculated to be 413, 507, and 556 mg/HD, respectively (Table 2). Subsequently, all references to Met treatments are identified by these mg/HD Met intake levels. Feed consumption for the 413 and 507 mg/HD Met treatments, 110 and 108 g/HD, respectively, was significantly greater than the 105 g/HD intake of the 556 mg/HD Met level (Table 2). Due to the differences in feed intake among the treatments, all
other nutrients consumed by the hens in the 413 and 507 mg/HD Met treatments were elevated significantly compared to the 556 mg/HD Met treatment (Table 2). Conclusions based on the 556 mg/HD Met treatment may be confounded by effect of the additional nutrients. Egg production was significantly higher among hens fed 507 mg/HD Met compared to 556 mg/HD Met intake (Table 3). Egg production of hens fed 413 mg/HD Met was not significantly different from either other treatment. Egg weight was significantly increased for the 507 and 556 mg/HD Met levels compared to 413 mg/HD Met (Table 3).

Egg component yield was not significantly different among the levels of Met intake on a liquid percentage basis, yet was significant on a mass basis (Table 3). This mass increase corresponds to significant increases in egg weights from corresponding treatment groups. Previous investigations into the fortification of laying hen diets with Met have shown increases in egg weight (Martin et al., 1969). The 507 and 556 mg/HD Met treatments produced a significantly greater albumen weight than did 413 mg/HD Met (Table 3). Yolk weight was significantly higher at 556 mg/HD Met than at 413 mg/HD Met.

Albumen total solids were significantly higher at 556 mg/HD Met than at 413 mg/HD Met (Table 4). Albumen CP was significantly increased at 507 mg/HD MET compared to 556 mg/HD Met, which was significantly higher than 413 mg/HD Met. Yolk total solids were not significantly different among the treatments (Table 4). Yolk CP was significantly increased at 556 and 507 mg/HD Met compared to 413 mg/HD Met (Table 4). Observed increases in albumen total solids content compare favorably to the results reported by Carey et al. (1991) and Shafer et al. (1996). Additionally, albumen and yolk CP increases are in agreement with previous work of Shafer et al. (1996).

Angel cake heights of the 556 mg/HD Met treatment were significantly higher than those at 507 mg/HD Met. There were no significant differences in cake volume among the treatments (Table 5). There were no significant differences among treatments in sponge cake volume or height (Table 5). Emulsion separations at 60 min were not significantly different among the treatments (Table 5). Emulsion separation at 120 min was significantly increased for 556 mg/HD Met compared to 413 and 507 g/HD Met. The TPA of the albumen and yolk gelation plugs revealed no significant differences in albumen or yolk hardness or springiness (data not shown).

There were no significant differences in optical density of electrophoretic protein band images among all diets for both albumen and yolk (data not shown). Resolution of yolk proteins using the ND-PAGE produced poor resolution with a heavy background shadow that compromised image analysis, requiring manual selection for digital analysis by computer software. Background shadow may have been due to

### Table 3. Egg production, weight, and component yield as percentage of egg liquid and weight

<table>
<thead>
<tr>
<th>Methionine intake (mg/HD)</th>
<th>Egg production (%)</th>
<th>Egg weight (g)</th>
<th>Albumen Liquid (%)</th>
<th>Albumen Weight (g)</th>
<th>Yolk Liquid (%)</th>
<th>Yolk Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>413</td>
<td>83.7</td>
<td>63.7</td>
<td>68.5</td>
<td>38.5</td>
<td>31.5</td>
<td>17.5</td>
</tr>
<tr>
<td>507</td>
<td>85.7</td>
<td>65.1</td>
<td>68.6</td>
<td>39.7</td>
<td>31.4</td>
<td>18.0</td>
</tr>
<tr>
<td>556</td>
<td>83.0</td>
<td>65.6</td>
<td>68.6</td>
<td>39.9</td>
<td>31.4</td>
<td>18.1</td>
</tr>
</tbody>
</table>

**Notes:**
- A,B Means within a column with no common superscript differ significantly (*P* < 0.01).
- HD = per live hen per day.

### Table 4. Egg component solids and crude protein content

<table>
<thead>
<tr>
<th>Methionine intake (mg/HD)</th>
<th>Albumen Solids (%)</th>
<th>Albumen Protein (%)</th>
<th>Yolk Solids (%)</th>
<th>Yolk Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>413</td>
<td>11.1</td>
<td>9.5</td>
<td>50.2</td>
<td>16.9</td>
</tr>
<tr>
<td>507</td>
<td>11.3</td>
<td>10.1</td>
<td>51.1</td>
<td>17.5</td>
</tr>
<tr>
<td>556</td>
<td>11.5</td>
<td>9.8</td>
<td>50.7</td>
<td>17.2</td>
</tr>
</tbody>
</table>

**Notes:**
- A-C Means within a column with no common superscript differ significantly (*P* < 0.01).
- HD = per live hen per day.
lipids separating as lipoproteins migrated through the resolving gel.

Body weight was not significantly different among the treatments. Initial and final BW were 1,539 and 1,528 g, respectively (data not shown). Mortality rates were not significantly different among Met intake levels and averaged 0.11% for all treatments (data not shown).

**DISCUSSION**

These findings indicate that Met supplementation rate may be an important factor in elevating the total solids and CP content of eggs being produced for the LE market. Functionality, TPA, and production parameters were not adversely affected by Met intake level.

Results from this experiment correlate with previous investigations by Carey et al. (1991) and Shafer et al. (1996), and suggest higher levels of Met supplementation may influence egg component yield, solids, and CP content without adversely affecting EP, weight, functionality, or mortality rate.

As more layer production is dedicated to LE markets, the impact of amino acid supplementation on EP and yield may offer an efficient and economical management technique for modification of egg component solids and protein content. Enhanced solids and protein content allows a LE producer to produce a greater volume of LEP from fewer eggs, thereby reducing production expenses while producing the same quantity of LE. Nutritional manipulation of egg component composition by amino acids, and their analogs, may generate economic and production advantages for the LE industry.

Commercial shell egg industry productivity has increased with the advent of economical amino acid feed supplements. The LE industry will accrue additional benefits through economical amino acid supplementation if internal egg composition can be improved. Albumen and yolk protein constituents may offer opportunity for nutritional manipulation of composition, as related to mass and solids content. Changes in these factors may affect whole egg weight and production parameters. Albumen and yolk components respond independently to nutritional changes due to unique protein synthesis mechanisms for each. Cellular biological factors that regulate protein synthesis need further investigation to elucidate the specific roles of individual amino acids.

Future investigations may expand the understanding of nutritional influences on egg component composition. Product opportunities may exist for EP having increased or enhanced protein content. With nutritional modification, egg enhancement may initiate at the layer production complex and continue throughout processing. Protein and solids alteration may influence lipoprotein, lipid, and water content, or possibly lipid to protein ratio within the egg. These studies indicate that changes in total solids and CP can occur independent of egg size changes. The implications of internal egg modification occurring independently from egg size may prove beneficial to the LE industry via utilization of eggs falling in low demand size categories. Thus, production may be aligned for both the shell and LE markets. The end state may be lipid content modification with an accompanying cholesterol content alteration.

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**REFERENCES**


albumen, yolk, and centrifuged whole egg. J. Food Sci. 35:774–778.


