Implication of milling methods, thermal treatment, and particle size of feed in layers on mineral digestibility and retention of minerals in egg contents

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ABSTRACT Feed production with different milling methods, thermal treatment, and particle size may influence mineral digestibility and retention in eggs. The present study investigated the impact of roller (R) and hammer (H) mills, mash (M) and expandate (E) with fine (F) and coarse (C) particle sizes, on apparent ileal absorption (AIA) and apparent total digestibility (ATD) and retention of calcium, phosphorus, magnesium, zinc, manganese, copper and iron in yolk, albumen, and shell. A total of 384 hens (Lohmann Brown), 19 weeks old, were assigned using a randomized design with a $2 \times 2 \times 2$ factorial arrangement. Eight experimental diets were offered ad libitum during the whole experimental period and one week before for diet adaptation. The AIA of magnesium, zinc, copper, and iron was higher in treatment R in comparison with treatment H ($P < 0.01$, $P \leq 0.03$, $P < 0.01$ and $P < 0.01$, respectively). The AIA of magnesium was higher in treatment M than treatment E ($P < 0.01$). The AIA of magnesium was higher in treatment C in comparison with treatment F ($P \leq 0.05$) due to particle size. The ATD of copper and iron was higher in treatment R than treatment H ($P < 0.01$ and $P \leq 0.03$, respectively). The ATD was higher for phosphorus and lower for iron in treatment F than treatment C ($P \leq 0.05$ and $P \leq 0.02$, respectively). The copper concentration in yolk and albumen was higher in treatment C than treatment F ($P < 0.01$ and $P \leq 0.03$, respectively). Besides a few overall interactions, the AIA and ATD of copper and manganese were lower in H+M group than R+M group ($P \leq 0.05$). The ATD of iron was higher in the M+C group compared to the M+F group ($P < 0.01$), whereas the albumen zinc concentration was higher in the E+C group than E+F group ($P < 0.01$). In conclusion, the feed produced by hammer mill had negative effects on AIA and ATD for trace elements in particular, but mineral concentrations in egg contents were mostly comparable for all treatments. Therefore, milling methods, thermal treatment, and particle sizes used in the present study can be used for layer feed formulation without negatively affecting egg quality.

Key words: feed production, poultry, hammer mill, roller mill, expandate

INTRODUCTION

Mineral nutrition is an important aspect for optimal egg production. In general, minerals and trace elements play major roles in vital physiological functions, bone development, and egg formation in poultry. The provision of optimum mineral content in the feed is essential for the production of quality eggs (Leeson and Summers, 2001; Mabe et al., 2003). Minerals are transferred into egg contents through blood, either in ionic form like sodium and potassium or in complex structures with lipoproteins as calcium, iron and zinc (Larbier and Leclercq, 1992). Dietary trace elements make a significant contribution to egg formation. The zinc status of the hen is directly related to activity of carbonic anhydrase.
(a calcium binding protein), which is essential for egg shell formation. Moreover, the hatchability and embryonic development may adversely be affected due to inadequate intake of dietary zinc (Hudson et al., 2004). The decline in shell quality due to lack of zinc in the saline water supply was counteracted by zinc supplementation, which increased the carbonic anhydrase level resulting in less shell defects (Leeson and Summers, 2001). For optimum egg production and quality, the hen’s requirements for critical minerals, including manganese, needs to be met (Tang et al., 2006). The addition of manganese in the diet decreased the number of cracked eggs, and adding manganese with zinc increased shell strength (Essary and Holmes, 1964) and reduced number of broken eggs (Zamani et al., 2005). The copper deficiency in the laying hen resulted in the production of eggs of abnormal size and shape, wrinkled and rough textured shells, and an increase in shell-less eggs (Baumgartner et al., 1978). Almost all of the yolk iron binds to phosvitin, a phosphoprotein known as an iron-carrier in egg yolk (Ishikawa et al., 2004).

In addition to the mineral content’s role in egg formation, it is equally important for breeders. The nutrient composition of fertile eggs has a strong influence on nutrient uptake by the embryo (Uni et al., 2012). The deficiency of calcium and phosphorus in general, and copper, manganese, and zinc in particular, may cause skeletal, immune, and cardiovascular system disorders, in addition to reduced hatchability and increased embryonic mortality (Angel, 2007; Dibner et al., 2007; Uni et al., 2012).

The use of roller and hammer mills for the reduction of feed particle size is common in the poultry feed industry (Koch, 2002; Amerah et al., 2007). The roller mill requires less energy, produces cubic or rectangular shaped (Koch, 2002) particle that are more uniform in size (Amerah et al., 2007) than hammer mill, which is easier to use (Koch, 2002), and produces more spherical shaped particles but a greater amount of fines (Reece et al., 1985).

Particle size distribution in feed affects egg quality and body weight, with small particle bound nutrients less effectively utilized (Tang et al., 2006). Chickens have a preference for larger feed particles (Schiffman, 1968) at all ages (Portella et al., 1988), but vitamins and minerals are mainly contained in smaller particles. The particle size is more critical in mash diets as compared to pelleted or crumbled diets, due to the selective feed intake of birds. Coarse particles require a longer transit time, which may allow for increased mineral digestion and absorption (Amerah et al., 2007). The utilization of calcium, total phosphorus, and phytate phosphorus was higher in broilers fed diets with coarse maize particle size (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Moreover, feeding of coarse particle size diet improved bone ash and plasma phosphorus levels in broilers (Carlos and Edwards Jr, 1997; Kilburn and Edwards, 2004).

Thermal treatment is commonly used in feed production to enhance apparent ileal digestibility of nutrients, to improve hygiene status of feed, and for the reduction of anti-nutritional factors (Mossel et al., 1967; Kilburn and Edwards, 2001; Peisker, 2006; Goodarzi Boroojeni et al., 2014b). Heat treatment was shown to influence bacterial status of the crop (Goodarzi Boroojeni et al., 2014a) and improve feed conversion (Amerah et al., 2007). In comparison with long term thermal treatment, pelleting and expansion of broiler feed resulted in higher apparent ileal absorption of calcium, potassium, and sodium (Hafeez et al., 2014a). However, pelleting and crumbing of the diet may reduce bone ash and plasma mineral concentrations in broilers (Kilburn and Edwards, 2004), which might be due to degradation of larger particles into smaller ones during the pelleting process, which could minimize the transit time, an advantage of larger particles (Amerah et al., 2007).

According to our knowledge, no further data is available regarding the effect of the milling method, thermal treatment, and feed particle size on apparent ileal absorption and apparent total digestibility of minerals in layers. Furthermore, their effect on mineral and trace element concentrations in egg contents could also be of interest.

Therefore, the aim of the present study was to investigate the impact of milling methods including roller and hammer mills, thermal treatment including mash vs expansion, and feed particle size on the apparent ileal absorption and apparent total digestibility of minerals, and the concentrations of mineral and trace elements in yolk, albumen, and shell in laying hens.

**MATERIAL AND METHODS**

The animal trial was performed according to the guidelines of the Animal Welfare Act of Germany, and was approved by the local state office of occupational health and technical safety (Landesamt für Gesundheit und Soziales, LaGeSo, no. G 0117/11).

**Hens and Experimental Design**

The experiment lasted for 21 days. Three hundred and eighty four hens (Lohmann Brown) at the age of 19 weeks were used. The hens were obtained from a commercial pullet rearing farm where they were previously fed with mash feed produced with a hammer mill. Hens were kept in cages which consisted of an area for claw abrasion, a perch, and laying nests. Wood shavings were used as bedding material. The hens were divided into 8 experimental groups in 8 repetitions with 6 hens per pen (in total 48 hens per feeding group). The dimensions of the each cage were 2.15 m × 1.40 m, thereby giving each hen 0.5 m² or 5.4 ft² of space. Eight experimental diets produced from one identical basal diet (Table 1) met the nutritional recommendations by the
The experimental diets have been described in previous a publication Ruhnke et al., (2014). In brief, one identical basal experimental diet was produced according to the German Society of Nutrition Physiology (GfE, 1999), and were offered ad libitum during the whole experimental period and one week before for diet adaption. Each diet was supplemented with TiO₂ (Sigma Aldrich, St. Louis, MO) as an indigestible marker at 2 g/kg to analyze the apparent mineral absorption in the ileum and apparent total digestibility in the rectum (Short et al., 1996). The experiment was conducted using randomized design with a 3 way factorial arrangement. Experimental diets were formulated using roller mill (R) and hammer mill (H) as mash (M) and expandate (E) with coarse (C) and fine (F) particle size. The 8 different diets were formulated using a 2 × 2 × 2 arrangement and assigned to hens within 64 pens, with 8 pens per diet group.

### Diets for Wet Sieving Analysis

The wet sieving analysis of the experimental diets were performed as described by Ruhnke et al., (2014). In brief, a 100 g feed sample was soaked in 1 L water for 1 hour and sieved through nine sieves of different pore sizes (Analysensiebe, Retsch GmbH, Haan, Germany) using 10 L of water for 10 minutes under continuous shaking. The contents of the sieves were dried for 6 hours at 100°C before weighing and the dMEAN was calculated as described earlier (Fritz et al., 2012). The results are in Table 2.

### Data for Performance Parameters

Throughout the experimental period, data was collected for performance parameters, including laying performance, feed intake, FCR and body weight, which is explained in Ruhnke et al., (2014).

### Collection of Samples

At the end of experiment at 23 weeks of age, 3 hens per pen were randomly selected and killed after stunning by cervical dislocation followed by exsanguination. The digesta from the distal two-thirds of the ileum (without content in the distal 3 cm prior to ileo-cecal junction) and rectum was collected as described by (Kluth et al., 2005; Rezvani et al., 2008), pooled and freeze-dried until analysis for mineral contents. One day before slaughtering, three eggs per pen from 5 out of 8 repetitions for every diet group were randomly collected. After determining the egg quality variables which have been described in Hafeez et al., (2014b), the yolk, albumen, and shell content of all three eggs from the same pen were pooled and frozen at -20°C until further analysis for DM, ash, mineral, and trace elements concentrations.

### Apparent Ileal Absorption and Apparent Total Digestibility in Rectum

The apparent absorption of minerals and trace elements in the ileum and rectum was calculated using

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**Table 1.** Feed composition and nutrient content of the basal experimental diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>g/kg as fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>300.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>290.6</td>
</tr>
<tr>
<td>Soybean meal (42% CP)</td>
<td>224.8</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>86.0</td>
</tr>
<tr>
<td>Soy oil</td>
<td>44.6</td>
</tr>
<tr>
<td>Molasses</td>
<td>30.0</td>
</tr>
<tr>
<td>Mineral/Vitamin premix¹</td>
<td>12.0</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>7.8</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>1.0</td>
</tr>
<tr>
<td>L-Tryptophane</td>
<td>0.2</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Nutrient content g/kg dry matter as analyzed

| Dry matter                   | 881.9       |
| Crude protein                | 185.8       |
| Ether extract                | 59.7        |
| Crude fiber                  | 32.7        |
| Starch                      | 418.5       |
| Crude ash                    | 110.6       |
| Calcium                     | 35.7        |
| Phosphorus                   | 3.54        |
| Magnesium                    | 2.45        |
| Zinc                        | 0.078       |
| Manganese                   | 0.102       |
| Copper                      | 0.018       |
| Iron                        | 0.534       |
| Calculated ME (MJ/kg)        | 11.4        |

¹Mineral and vitamin premix (Spezialfutter Neuruppin, Neuruppin, Germany) containing per kg premix: 400 000 IU vitamin A; 40 000 IU vitamin D₃; 8 000 mg vitamin E (alpha-tocopherol acetate); 300 mg vitamin K₃; 250 mg vitamin B₁₂; 250 mg vitamin B₃; 2500 mg nicotinic acid; 400 mg vitamin B₆; 200 µg vitamin B₁₂; 25 000 µg biotin; 1000 mg calcium pantothenate; 100 mg folic acid; 8 000 mg choline chloride; 5000 mg zinc (zinc oxide); 2000 mg iron (iron carbonate); 6000 mg manganese (manganese oxide); 1200 mg copper (copper sulfate-pentahydrate); 45 mg iodine (calcium iodate); 30 mg cobalt (cobalt-(II)-sulfate-heptahydrate); 35 mg selenium (sodium selenite); 35 g sodium (sodium chloride); 55 g magnesium (magnesium oxide).
the following formula:

\[
\text{apparent absorption (\%)} = 100 - \left[ \frac{\text{concentration of marker in feed}}{\text{concentration of marker in digesta}} \times \frac{\text{concentration of nutrient in digesta}}{\text{concentration of nutrient in feed}} \right] \times 100.
\]

Analysis for Mineral Concentrations

The feed, ileal and rectal digesta, yolk, albumen, and shell samples were freeze dried, and the dry matter content was determined as a ratio of fresh matter weight to dried matter weight. The freeze dried samples were then ashed in a muffle furnace at 600°C for 6 h. The percent ash was determined in relation to DM in the respective sample. The method of atomic absorption spectrometry in an AAS Vario 6 spectrometer (Analytik Jena, Jena, Germany) was adopted to determine the concentrations of calcium, magnesium, iron, copper, manganese, and zinc. The concentration of phosphorus was determined with the ammonium vanadate/molybdate method as described by (Gericke and Kurmies, 1952). Concentrations of titanium dioxide in feed, ileum, and rectal digesta were determined according to the method described by (Short et al., 1996).

Data Analysis

For statistical analysis, the data were arranged in a three factorial arrangement (2 × 2 × 2) where milling methods (roller and hammer), thermal treatments (mash vs expandate), and particle size (coarse and fine) were subjected to ANOVA using GLM procedure. The analyses were performed to determine the main effects of treatment and their interactions. Tukey’s-b test was used as post-hoc test at \( P \leq 0.05 \) for grouping of treatment means. The normality was tested using Shapiro-Wilk. For statistical analysis, SPSS 20.0 (SPSS Inc., Chicago, IL) was used. The apparent ileal absorption and apparent total digestibility of minerals and their concentrations in yolk, albumen, and shell were measured on the basis of the pen as the experimental unit.

### RESULTS

In the present study, during the egg sampling week (d 15–21), the percent laying performance was not affected by the roller (89.1 ± 6.64) and hammer mill (91.7 ± 7.73), mash (90.6 ± 5.90) and expandate (90.7 ± 8.35), and the interaction between milling method and thermal treatment \( (P > 0.05) \). Moreover, feed particle size did not influence the percent laying performance \( (P > 0.05) \). Feed intake, FCR, and body weight were not affected by milling method, thermal treatment, and particle size \( (P > 0.05) \). The performance parameters are further discussed in Ruhnke et al., (2014).

Table 2 presents the results of the dry and wet sieving analysis. The expansion of feed had a pronounced effect on particle size after wet sieving. The difference between the coarse and fine particle sizes in the expansion groups is less pronounced after wet sieving in comparison with the mash groups. The impact of milling method, thermal treatment, and particle size on apparent ileal absorption of minerals is presented in Table 3. The AIA of Mg, Zn, Cu, and Fe was higher in treatment R in comparison with treatment H \((P < 0.01, P \leq 0.03, P < 0.01\text{ and } P < 0.01,\text{ respectively})\), whereas the AIA of Ca, P and Mn was not significantly different \( (P > 0.05) \). Thermal treatment affected the AIA of Mg, which was higher in treatment M than treatment E \((P < 0.01)\), but other variables were not affected \( (P > 0.05) \). Due to particle size, the AIA of Mg was higher in treatment C in comparison with treatment F \((P \leq 0.05)\). No effect of particle size was observed on the AIA of other minerals and trace elements \( (P > 0.05) \). The interaction between the milling method and thermal treatment influenced the AIA of Mn and Cu \((P \leq 0.02\text{ and } P < 0.01,\text{ respectively})\). The AIA of Mn within the R+M group \((19.7 ± 13.2)\) was significantly higher than that of the H+M group \((-3.79 ± 28.3)\). The R+E and H+E groups \((-2.3 ± 21.7\text{ and }-8.68 ± 23.4,\text{ respectively})\) had significantly higher interaction effects for AIA of Cu than the H+M group \((-36.9 ± 37.2)\) and significantly lower interaction effects than the R+M group \((37.5 ± 19.8)\). Thermal treatment and particle size had an overall interaction effect on the AIA of Cu and Fe \((P < 0.02, P \leq 0.05,\text{ respectively})\), however no differences were observed between groups. Table 4 shows the effect of the milling method, thermal treatment, and particle size of feed on apparent total digestibility of minerals and trace elements. The ATD of Cu and Fe

<table>
<thead>
<tr>
<th>Mill</th>
<th>Particle size</th>
<th>Thermal</th>
<th>Roller</th>
<th>Hammer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mash</td>
<td>Expandate</td>
<td>Mash</td>
</tr>
<tr>
<td>dMEAN after dry sieving analysis (mm)</td>
<td>1.93</td>
<td>2.43</td>
<td>1.28</td>
<td>1.27</td>
</tr>
<tr>
<td>dMEAN after wet sieving analysis (mm)</td>
<td>1.85</td>
<td>0.85</td>
<td>1.14</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Effect of milling method, thermal treatment and particle size of feed on percent apparent ileal absorption of minerals in layers.

<table>
<thead>
<tr>
<th>Mill</th>
<th>Thermal</th>
<th>Particle Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller</td>
<td>Thermal</td>
<td>Particle Size</td>
<td>P-value</td>
</tr>
<tr>
<td>50.0</td>
<td>49.1</td>
<td>46.8</td>
<td>0.85</td>
</tr>
<tr>
<td>45.2</td>
<td>35.3</td>
<td>35.1</td>
<td>0.98</td>
</tr>
<tr>
<td>40.0</td>
<td>32.0</td>
<td>39.9</td>
<td>0.01</td>
</tr>
<tr>
<td>18.6</td>
<td>8.50</td>
<td>17.1</td>
<td>0.03</td>
</tr>
<tr>
<td>13.1</td>
<td>2.79</td>
<td>7.97</td>
<td>0.07</td>
</tr>
<tr>
<td>17.7</td>
<td>-23.8</td>
<td>-0.111</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>30.3</td>
<td>6.65</td>
<td>22.5</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

1Data are means of 8 replicates per group.

Table 4. Effect of milling method, thermal treatment and particle size of feed on percent apparent total digestibility of minerals in layers.

<table>
<thead>
<tr>
<th>Mill</th>
<th>Thermal</th>
<th>Particle Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller</td>
<td>Thermal</td>
<td>Particle Size</td>
<td>P-value</td>
</tr>
<tr>
<td>52.3</td>
<td>46.1</td>
<td>44.1</td>
<td>0.16</td>
</tr>
<tr>
<td>31.1</td>
<td>35.4</td>
<td>32.7</td>
<td>0.20</td>
</tr>
<tr>
<td>43.8</td>
<td>39.5</td>
<td>40.0</td>
<td>0.40</td>
</tr>
<tr>
<td>19.4</td>
<td>11.7</td>
<td>14.8</td>
<td>0.16</td>
</tr>
<tr>
<td>6.50</td>
<td>-1.38</td>
<td>-1.14</td>
<td>0.02</td>
</tr>
<tr>
<td>18.1</td>
<td>-6.86</td>
<td>7.08</td>
<td>0.03</td>
</tr>
<tr>
<td>23.2</td>
<td>11.9</td>
<td>14.8</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1Data are means of 8 replicates per group.

was higher in treatment R than treatment H (P < 0.01 and P ≤ 0.03, respectively). The milling method had no influence on the ATD of other minerals and trace elements (P > 0.05). The thermal treatment of feed did not affect the ATD for any of the variables (P > 0.05) except Ca, which was higher in treatment E than treatment M (P < 0.01). The feed particle size affected the ATD of P which was higher in treatment F than treatment C (P < 0.05). The thermal treatment of feed particle size on yolk mineral and trace element concentrations are presented in Table 5. The DM and ash content were similar for all treatments (P > 0.05). The milling method did not affect the egg yolk concentration of any mineral or trace element under consideration (P > 0.05). Thermal treatment did not show any changes in yolk mineral and trace element levels (P > 0.05). The particle size influenced Cu concentration in the yolk, which was higher in treatment C than treatment F (P < 0.01). Zn concentration was affected by an overall interaction between milling method and particle size (P < 0.04), with no differences between groups. There were no other interaction effects observed (P > 0.05). Table 6 shows the response of albumen mineral and trace element concentrations to milling method, thermal processing, and feed particle size. There were no differences in DM and ash content due to all treatments (P > 0.05). The milling method did not affect mineral and trace element concentrations in albumen (P > 0.05). Due to thermal treatment, the concentration of Mn was higher in treatment M as compared to treatment E (P ≤ 0.04), while other variables were not affected (P > 0.05). The particle size affected albumen Cu concentrations, which...
were higher in treatment C than treatment F ($P \leq 0.03$), however the concentrations of other variables under consideration remained unchanged ($P > 0.05$). The interaction of thermal treatment and particle size affected the albumen Zn concentration ($P < 0.01$), which was higher in E+C group (1.89 ± 0.03) in comparison with the E+F group (0.98 ± 0.42). An overall interaction between thermal treatment, milling method, and particle size affected albumen Fe concentration ($P < 0.03$) with no differences between groups. The DM, ash content, and mineral and trace element concentrations in egg shells were not affected ($P > 0.05$) by milling method, thermal treatment, and particle size (Table 7). The concentration of Mn in the shell was affected by an overall interaction between thermal treatment and particle size ($P \leq 0.02$) without any differences between groups.

### DISCUSSION

In the present study, the milling method influenced some of the egg quality parameters. The yolk index and yolk height were higher and shell membrane weight and percent shell membrane weight were lower in hammer mill treatment as compared to roller mill treatment. Expansion of the feed displayed a higher percent shell membrane weight in comparison with mash treatment. The albumen height, shell thickness, and shell weight were higher, whereas shell density was lower in hammer mill treatment compared to fine. However most of the economically important indicators for exterior and interior egg quality were comparable among the treatments, which are explained in Hafeez et al. (2014b).

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**Table 5. Effect of milling method, thermal treatment and particle size of feed on yolk minerals in layers.**

<table>
<thead>
<tr>
<th>Mill</th>
<th>Thermal</th>
<th>Particle Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller</td>
<td>Thermal</td>
<td>Coarse</td>
<td>SEM</td>
</tr>
<tr>
<td>Hammer</td>
<td>Thermal</td>
<td>Fine</td>
<td></td>
</tr>
<tr>
<td>Mash</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Expandate</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Coarse</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Fine</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
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<tr>
<td>SEM</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ash (% of DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ca g/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>P g/kg (DM)</td>
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<td>SEM</td>
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</tr>
<tr>
<td>Mg g/kg (DM)</td>
<td></td>
<td>SEM</td>
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</tr>
<tr>
<td>Zn mg/kg (DM)</td>
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<td>SEM</td>
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</tr>
<tr>
<td>Mn mg/kg (DM)</td>
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</tr>
<tr>
<td>Cu mg/kg (DM)</td>
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<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Fe mg/kg (DM)</td>
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<td>SEM</td>
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</tr>
<tr>
<td>Data are means of 5 replicates per group.</td>
<td></td>
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</tr>
</tbody>
</table>

**Table 6. Effect of milling method, thermal treatment and particle size of feed on albumen minerals in layers.**

<table>
<thead>
<tr>
<th>Mill</th>
<th>Thermal</th>
<th>Particle Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller</td>
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<td>Coarse</td>
<td>SEM</td>
</tr>
<tr>
<td>Hammer</td>
<td>Thermal</td>
<td>Fine</td>
<td></td>
</tr>
<tr>
<td>Mash</td>
<td>Thermal</td>
<td>SEM</td>
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<tr>
<td>Expandate</td>
<td>Thermal</td>
<td>SEM</td>
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</tr>
<tr>
<td>Coarse</td>
<td>Thermal</td>
<td>SEM</td>
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</tr>
<tr>
<td>Fine</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ash (% of DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ca g/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>P g/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Mg g/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Zn mg/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
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<tr>
<td>Mn mg/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Cu mg/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Fe mg/kg (DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Data are means of 5 replicates per group.</td>
<td></td>
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</tr>
</tbody>
</table>

**Table 7. Effect of milling method, thermal treatment and particle size of feed on shell minerals in layers.**

<table>
<thead>
<tr>
<th>Mill</th>
<th>Thermal</th>
<th>Particle Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roller</td>
<td>Thermal</td>
<td>Coarse</td>
<td>SEM</td>
</tr>
<tr>
<td>Hammer</td>
<td>Thermal</td>
<td>Fine</td>
<td></td>
</tr>
<tr>
<td>Mash</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Expandate</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Coarse</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Fine</td>
<td>Thermal</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ash (% of DM)</td>
<td></td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Ca g/kg (DM)</td>
<td></td>
<td>SEM</td>
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<tr>
<td>P g/kg (DM)</td>
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<tr>
<td>Mg g/kg (DM)</td>
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<tr>
<td>Data are means of 5 replicates per group.</td>
<td></td>
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</tbody>
</table>
The discrete mean (dMEAN) obtained by dry sieving analysis of particle size is similar to the macro-structure of feed, whereas the dMEAN obtained by wet sieving analysis of particle size is similar to the microstructure of the feed. The macro-structure of the feed is converted to micro-structure in the upper part of digestive tract of the bird due to salivary and enzymatic action. Therefore, mash feed may be characterized on the basis of dry sieving analysis whereas expanded feed may be characterized by either dry or wet sieving (Svihus, 2006). Our results indicated that after wet sieving analysis, which involves dissolving feed in water, the differences between coarse and fine particles were less pronounced in expanded groups as compared to mash groups. Therefore, it might be considered that the feed intake may be affected by macro-structure of the feed, however, the gut function may be influenced by micro-structure of feed in the digestive tract (Svihus, 2006).

The milling methods showed some interesting effects regarding mineral and trace element digestibility. The apparent ileal absorption of magnesium and trace elements including zinc, copper and iron was reduced in the feed ground by the hammer mill as compared to roller mill. Despite producing irregular, cubic, or rectangular shaped particles (Koch, 2002), the roller mill generates a more uniform sized particles in comparison with hammer mill (Amerah et al., 2007), which produces spherical shaped particles with a greater amount of fine particles (Reece et al., 1985). Due to the fact that the coarser grinding of grains to a more uniform particle size may improve the performance of mature birds due to more gizzard development leading to increased grinding, gut motility, and nutrient digestion (Amerah et al., 2007), the production of uniform sized particles by roller mill may have resulted in higher apparent ileal absorption of magnesium and trace elements. A recent study revealed that the particle size distribution affected egg quality and body weight as small particle nutrients were not effectively utilized (Tang et al., 2006). Furthermore, our results indicate that ileal absorption of trace elements is more critical than that of minerals affected by different milling methods. The apparent total digestibility of copper and iron was higher in feed produced by roller mill as compared to hammer mill, while other variables remained unchanged. The minerals and trace elements are mostly absorbed in the ileum and jejunum (Leeson and Summers, 2001). The apparent total digestibility results show that lower absorption for magnesium and trace elements in ileum was compensated by absorption in the hind gut. Furthermore, the mineral and trace element concentrations in yolk, albumen, and shell were comparable. It suggests that the effect of the milling method in decreasing absorption of magnesium and trace elements was limited to the small intestine, and that the body reserves and absorption in the hind gut were sufficient to offset such effects, thereby ensuring the provision of optimum amounts of mineral and trace elements into egg contents.

Thermal treatment of feed did not affect the AIA and ATD of any of the mineral or trace elements, except a higher ATD of calcium and lower apparent ileal absorption of magnesium was observed in birds fed expanded compared to mash feeding. A recent study showed that the expansion of broiler diets increased the AIA of calcium in comparison with long term heat treatment, whereas phosphorus, magnesium, zinc, manganese, copper and iron were comparable (Hafeez et al., 2014a). Similar data was presented by Iji et al., (2003), revealing that absorption of calcium was increased in broilers by oven drying fresh maize grains for 24 hours at 85°C and 95°C. However, the phosphorus absorption was also increased at 85°C. In contrast, another study indicated that the pelleting of a maize diet reduced phytate phosphorus retention (Kilburn and Edwards, 2001). In the present study, the AIA and ATD of phosphorus was not affected by thermal treatment. Both organic (corn and wheat) and inorganic (monocalcium phosphate) sources of phosphorus were included in the layer diet. The phosphorus availability in different diet ingredients has substantial variability (Rodehuts cord et al., 2012). Cereals contain 60–70% phytic acid, which is 0–50% available, whereas monocalcium phosphate contains 98% available phosphorus (Rutherford et al., 2002). Therefore, the presence of both phytic acid and monocalcium phosphate might be the reason that the AIA and ATD of phosphorus were comparable. The concentrations of all minerals and trace elements in yolk, albumen, and shell were comparable, except that of albumen manganese, which was higher in hens fed mash compared to those fed expanded feed. Manganese is largely stored in yolk and albumen only contains traces (Leeson and Summers, 2001; Uni et al., 2012). Therefore, the different concentration of manganese in albumen may not have any meaningful physiological consequences on egg quality.

The feed particle size did not affect most of the minerals in egg contents but had some interesting correlations for their AIA and ATD. The AIA for magnesium was higher in birds fed with coarse feed particles than fine. Additionally in this study, the egg shell thickness and shell weight was higher in coarse particle size treatment than fine treatment, which is discussed in Hafeez, et al., (2014b). Due to the fact that magnesium plays an important role in egg shell formation (Leeson and Summers, 2001), the higher apparent ileal digestibility of magnesium with increasing particle size likely resulted in higher shell formation in terms of shell thickness and shell weight. The ATD was higher for phosphorus and lower for iron in birds given feed of fine particle size compared to those given coarse feed. In general, a reduction in particle size increases the surface area for enzymatic activity, modifies the physical characteristics of feed, and may result in improved animal performance (Waldroup, 1997; Goodband et al., 2002). However, some recent studies emphasize the positive effect of coarse feed particles on starch availability (Svihus et al., 2010).
Amerah et al., (2007) argued that coarse particles may result in higher mineral digestion and absorption due to the possibility of a longer transit time. Other data suggest that coarse maize particles resulted in a higher utilization of calcium, total phosphorus, and phytate phosphorus in broilers (Kasim and Edwards, 2000; Kilburn and Edwards, 2001). Further findings show that feeding of coarse particle size diets improved bone ash and plasma phosphorus levels in broilers (Carlos and Edwards Jr, 1997; Kilburn and Edwards, 2004).

The interaction between milling methods and thermal treatment resulted in significant differences in AIA and ATD of copper and manganese. For copper, the mash feed produced by roller mill resulted in a higher AIA and ATD than expanded feed and mash feed produced by hammer mill. A similar trend was observed for manganese, where mash feed produced by roller mill resulted in a significantly higher AIA and ATD compared to hens fed mash feed produced by hammer mill. Therefore, the combination of hammer mill and mash resulted in significantly lower digestibility than roller mill and mash for both copper and manganese. The literature suggests that roller mill produces more uniform sized particles as compared to hammer mill (Amerah et al., 2007), which produces a greater amount of fine particles (Reece et al., 1985). The particles produced by hammer mill in mash form had more uniformity problems than those subjected to expansion. Furthermore, the particles produced by roller mill in mash and expanded form had a more uniform size than those produced by hammer mill, especially in mash form. Due to the fact that a more uniform particle size may improve the performance of mature birds (Amerah et al., 2007), the lower AIA and ATD for both copper and manganese for the interaction effect, was possibly caused by the problem of uniformity of particles produced by hammer mill in mash form, as compared to other combinations of milling type and thermal treatment.

The interaction between thermal treatment and particle size affected the retention of some trace elements in egg contents. Our results show that coarse particle size in combination with both the mash and expandate forms resulted in a higher albumen zinc concentration than fine feed in the expandate form. Both interactions show that the coarse particle size in combination with both the mash and expandate forms increased the retention for couple of trace elements in comparison with combinations of fine feed particles with mash and expandate forms. However, due to the fact that interaction between thermal treatment and particle size had no effect on AIA, ATD, and the retention of most of the minerals and trace elements in egg contents, these effects might have no meaningful biological consequences. No further data was reported in literature, to our knowledge, regarding the effect of milling methods, thermal treatments, particle size and their interaction on mineral digestibility and egg mineral contents.

In general, yolk has higher concentrations of calcium, phosphorus, manganese, iron, copper, and zinc as compared to albumen, and the shell is an important source of calcium and magnesium release during embryonic development (Uni et al., 2012). In the present study, out of total mineral concentrations retained in yolk and albumen, the yolk contained approximately 77% calcium, 90% phosphorus, 98% zinc, 94% manganese, 78% copper, and 91% iron. Our results regarding ratios of mineral and trace elements contained in yolk and albumen are in agreement with Uni et al., (2012), who reported approximately 83% calcium, 92% phosphorus, 65% zinc, 96% manganese, 77% copper, and 88% iron in yolk as compared to albumen.

In conclusion, feed produced by hammer mill had a negative effect on AIA and ATD for trace elements in particular, which could be critical due to their major role in various biochemical processes in the chicken's body. However, the mineral and trace element concentrations in egg yolk, albumen, and shell, the performance parameters and egg quality indicators of economic interest, were mostly comparable for all the treatments. Therefore, any combination of milling methods, thermal treatments, and particle sizes used in the present study may be used for feed production of laying hens.

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REFERENCES


