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Effects of a Pre-Molt Calcium and Low-Energy Molt Program on Laying Hen Physiology

Authors
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Summary and Implications
The objectives of this study were to compare stress measures and bone quality of laying hens when offered a Ca pre-molt treatment followed by low-energy molt diets versus a traditional feed withdrawal before, during, and after an induced molt. A total of 189 Hy-Line W-36 laying hens (85 wk of age, 1.7 ± 0.2 kg), housed 3 per cage, (413 cm²/hen) were used. Six treatments were compared in a 2 × 3 factorial design with 2 Ca (coarse and fine) pre-molt treatments (coarse and fine) and 3 molt diets: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM). The Ca pre-molt treatment was defined as the period when the hens received either a combination of fine (0.14 mm in diameter) and coarse (2.27 mm in diameter) CaCO₃ or an all-fine CaCO₃ mixed into a commercial diet for 1 wk. Both diets were formulated to contain 4.6% Ca, such that only the particle size of the CaCO₃ differed between the 2 treatments. Hens had free access to feed and water and had a 24-h photoperiod. The 3 molt diets were applied for a total of 28 d. The hens assigned to the FW molt diet were deprived of feed for 7 d with free access to water followed by 21 d of skip-a-day feeding restricted to 60 g of feed / hen per feeding day. The hens fed the WM and SH molt diets were provided free access to feed and water during the entire 28 d molt period. Lighting was reduced to 8 h for the first 3 wk and was then increased to 12 h at the start of the last week of molt. During the 22 wk post-molt, hens were fed a laying hen diet and lighting was increased by 1 h each week to 16 h. None of the treatments resulted in an increased heterophil to lymphocyte ratio during or post-molt compared to baseline values, which would have suggested increased stress in the laying hen. Additionally, any changes reported during molt in bone quality returned to baseline values during the post-molt period. Therefore, these treatments are acceptable for inducing molt in the laying hen.

Introduction
In commercial laying hens, molt is induced to allow for a second laying cycle, extending the productive life of the hen. During molt, egg production ceases and the reproductive tract regresses. Molt has been traditionally induced by a period of feed withdrawal (FW). However, this practice has recently raised concern for the well-being of the laying hen. In addition, industry groups have recommended that after January 1, 2006, producers implement only non-fasting molt programs. Previous research has reported the effectiveness of low-energy diets as alternatives to FW for inducing molt. In addition, a diet severely deficient in Ca has also been used to induce molt. The form of Ca is also important with particulates (coarse Ca) solubilized more slowly from the digestive tract compared to powdered Ca (fine Ca). The coarse Ca may provide the hen with more Ca for use in eggshell and bone formation. Therefore, a fine-Ca pre-molt treatment may result in a more efficient molt and its possible effects on stress and bone quality were examined. The objectives of this study were to compare stress measures and bone quality of laying hens when offered a Ca pre-molt treatment followed by low-energy molt diets versus a traditional FW before, during, and after an induced molt.

Materials and Methods
Animals and Location: A total of 189 Hy-Line W-36 laying hens (85 wk of age), weighing 1.7 ± 0.2 kg, were used in this study. Research was conducted over 29 wk from July 2007 to February 2008 at the Iowa State University Poultry Research Center in Ames, IA. The project was approved by the Iowa State University Animal Care and Use Committee.

Diets, Housing and Husbandry: Laying hens were housed 3 per cage (30.5 cm wide × 40.6 cm deep × 44.5 cm high), providing 413 cm²/hen. Wire flooring was used in all cages and each cage was equipped with a plastic self-feeder and a nipple drinker. All cages were located in 2 identical, light-controlled fan-ventilated rooms.

Treatments: Six treatments were compared in a 2 × 3 factorial arrangement with 2 Ca (fine and coarse) pre-molt treatments and 3 molt diets: FW, soybean hulls (SH), and wheat middlings (WM). The Ca pre-molt treatment was defined as the period when the hens received either a combination of fine (0.14 mm in diameter) and coarse (2.27 mm in diameter) CaCO₃ or an all-fine CaCO₃ mixed into a commercial diet for 1 wk. Both diets were formulated to contain 4.6% Ca, such that only the particle size of the CaCO₃ differed between the 2 treatments. Hens had free access to feed and water and had a 24-h photoperiod. The 3 molt diets were applied for a total of
hens were euthanized by CO2 asphyxiation. Fresh weights
were determined and plasma Ca and inorganic P concentrations and alkaline
phosphatase activity (ALP). After blood was collected, all
plasma was collected for analysis of
lymphocyte (H:L) ratio. The remaining blood was
treatments). Blood smears were made for the heterophil to
the post-molt period (9 hens from each of the 6
treatments), and at the end of
molt period (9 hens from each of the 2
treatments). Blood smears were made for the heterophil to
lymphocyte (H:L) ratio. The remaining blood was
centrifuged and the plasma was collected for analysis of
plasma Ca and inorganic P concentrations and alkaline
phosphatase activity (ALP). After blood was collected, all
hens were euthanized by CO2 asphyxiation. Fresh weights
of ovaries and oviducts were recorded and eggs in the
reproductive tract, if any, were removed before weighing.
The left-side humerus and femur bones were used to
determine bone mineral content. Ash content was
expressed as a percentage of the dry bone weight.

Physiologic Parameters: Blood was collected from each
hen at the end of the baseline period (9 hens), at the end of
the Ca pre-molt treatment (9 hens from each of the 2
treatments), during the middle and end of the molt period (9 hens from each of the 6 treatments), and at the end of
the post-molt period (9 hens from each of the 6
treatments). Blood smears were made for the heterophil to
lymphocyte (H:L) ratio. The remaining blood was
centrifuged and the plasma was collected for analysis of
plasma Ca and inorganic P concentrations and alkaline
phosphatase activity (ALP). After blood was collected, all
hens were euthanized by CO2 asphyxiation. Fresh weights
of ovaries and oviducts were recorded and eggs in the
reproductive tract, if any, were removed before weighing.
The left-side humerus and femur bones were used to
determine bone mineral content. Ash content was
expressed as a percentage of the dry bone weight.

Statistical Analysis: The experimental design was a
randomized complete block design with treatments in a 2 × 3 factorial arrangement with 2 Ca pre-molt treatments and 3 molt diets. The experimental unit was the individual
hen (n = 189). The baseline model included treatment and
block (based on initial body weight and cage location
within the barn; 1 to 11). During the Ca pre-molt
treatment, Ca treatment and block were used in the model. During and post-molt, the model included Ca pre-molt
treatment, molt diet, the 2-way interaction of these, and
block. The effect of the Ca pre-molt treatment was
assessed using the main effect of the Ca treatment from
the ANOVA table, whereas the effect of the molt diet was assessed by Fisher’s least significant difference.
Experimental values were compared to baseline values
using Dunnett’s test. A P < 0.05 was considered
significant.

Results and Discussion
Pre-molt calcium treatment: Hens fed the coarse-Ca pre-
molt treatment had higher ovary and oviduct weights
compared to hens during the baseline period (P < 0.05).

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28 d. The hens assigned to the FW molt diet were
deprived of feed for 7 d with free access to water
followed by 21 d of skip-a-day feeding restricted to 60 g
of feed/hen per feeding day. The hens fed the WM and
SH molt diets were given free access to feed and water
during the entire 28 d molt period. Lighting was reduced to
8 h for the first 3 wk and was then increased to 12 h at
the start of the last week of molt. During the 22 wk after
molt, hens were provided with laying hen diets. This
period was divided into the first 2 wk after molt and the
following 20 wk according to diet recommendations from
the 2007–2008 Hy-Line W-36 commercial management
guide. Hens were given free access to feed and water and
the lighting was increased by 1 h each week until reaching
a 16 h photoperiod.

During molt: Hens assigned to the 6 treatments had lower
ovary and oviduct weights and plasma Ca concentrations,
and higher ALP activity compared to hens during the
baseline period (P < 0.05). There were no differences (P >
0.05) between baseline values and Ca pre-molt treatment
values in H:L ratios, humerus-ash percentages, or plasma
inorganic P concentrations (Tables 1 and 2). When
comparing hens fed the 2 Ca pre-molt treatments, there
were no differences in any of the measures (P > 0.05).
Hens fed the FW and WM molt diets had lower femur-ash
percentages compared to hens during the baseline period.
When comparing hens fed the 3 molt diets, hens fed the
WM molt diet had higher ovary weights compared to hens
during the baseline period, but there were no differences
among the 3 molt diets (Tables 1 and 2).

Post-molt: Hens assigned to the Ca pre-molt treatments
had no differences compared to hens from the baseline
period in ovary weights, bone-ash percentages, H:L ratios,
plasma Ca, or inorganic P concentrations (P > 0.05).
However, hens fed the fine-Ca pre-molt treatment had
higher ALP activity compared to hens during the baseline
period and higher oviduct weights compared to the
baseline period and to the coarse-Ca pre-molt treatment
(P < 0.05). Hens fed the molt diets had no differences
compared to hens from the baseline period in ovary and
oviduct weights, bone-ash percentages, H:L ratios, plasma
Ca, or inorganic P concentrations (P < 0.05). Hens fed the
SH molt diet had higher ALP activity compared to hens
during the baseline period, but there were no differences
in any of the measures among the 3 molt diets (Tables 1
and 2).

Conclusions: The fine-Ca pre-molt treatment did not
negatively affect bone quality of the laying hen and did
not result in an increased H:L ratio compared to baseline
values, which would have suggested a stress effect on the
laying hen. The 3 molt diets also had no detrimental
effects on stress and bone quality of the laying hen. Any
differences that were reported during molt returned to
baseline values during the post-molt period. Therefore,
these treatments are acceptable for use during an induced
molt in the laying hen.
Acknowledgements

We would like to thank the Midwest Poultry Research Program (St. Paul, MN), the Iowa Egg Council (Urbandale, IA), and ILC Resources (Des Moines, IA) for their financial support. Additionally, we would like to acknowledge ADM (Des Moines, IA), Evonik Degussa Corporation (Kennesaw, GA), DSM Nutrition (Ames, IA), Feed Energy Company (Des Moines, IA), ILC Resources (Alden, IA), and Sparboe Farms (Litchfield, MN) for in-kind contributions. We are grateful to the personnel in the Bregendahl and Johnson laboratories and we thank Jeff Tjelta and Bill Larson at the Iowa State University Poultry Science Research Center for their cooperation and support.
Table 1. Comparison of responses of hens during each period for reproductive tract weights and bone ash-percentages

<table>
<thead>
<tr>
<th>Measures</th>
<th>Baseline</th>
<th>Coarse</th>
<th>Fine</th>
<th>FW</th>
<th>SH</th>
<th>WM</th>
<th>SEM</th>
<th>Ca</th>
<th>Molt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary, g</td>
<td>41.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.95</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>50.9*</td>
<td>49.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.16</td>
<td>0.73</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>5.92*</td>
<td>5.60*</td>
<td>5.27*&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>4.51*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.66</td>
<td>0.74</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Post-molt</td>
<td>42.3</td>
<td>48.3</td>
<td>45.8</td>
<td>42.9</td>
<td>47.3</td>
<td>3.62</td>
<td>0.06</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Oviduct, g</td>
<td>52.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.19</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>66.4*</td>
<td>64.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.10</td>
<td>0.67</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>13.2*</td>
<td>12.2*</td>
<td>10.9*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.2*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.08</td>
<td>0.60</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Post-molt</td>
<td>52.4</td>
<td>65.4*</td>
<td>61.2</td>
<td>55.1</td>
<td>60.3</td>
<td>4.09</td>
<td>0.001</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Humerus bone, %</td>
<td>64.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.68</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>62.0</td>
<td>61.0*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.43</td>
<td>0.52</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>61.0</td>
<td>61.7</td>
<td>61.7</td>
<td>60.8</td>
<td>61.7</td>
<td>0.96</td>
<td>0.36</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Post-molt</td>
<td>62.2</td>
<td>61.5</td>
<td>61.7</td>
<td>61.6</td>
<td>62.3</td>
<td>1.50</td>
<td>0.85</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Femur bone, %</td>
<td>55.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.42</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>55.1</td>
<td>54.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.02</td>
<td>0.75</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>51.5*</td>
<td>50.5*</td>
<td>50.9*</td>
<td>51.7</td>
<td>50.3*</td>
<td>1.06</td>
<td>0.22</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Post-molt</td>
<td>52.9</td>
<td>53.3</td>
<td>53.2</td>
<td>52.5</td>
<td>53.6</td>
<td>1.55</td>
<td>0.72</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are least squares means ± pooled SEM (n = 9).
2 Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) CaCO<sub>3</sub> or as an all-fine CaCO<sub>3</sub> mixed into a laying hen diet for a 1 wk pre-molt Ca treatment.
3 Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
4<sup>P</sup>-values from main effect of Ca pre-molt treatment or molt diet.
5<sup>a,b</sup> Means within a row lacking a common superscript differ (P < 0.05).
6<sup>*</sup> Means within a row differ from baseline value (P < 0.05). <sup>*</sup>P-value from Dunnett’s comparison.
## Table 2. Comparison of responses of hens during each period for the blood measures

<table>
<thead>
<tr>
<th>Measures</th>
<th>Treatments</th>
<th>Calcium pre-molt</th>
<th>Molt</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Coarse</td>
<td>Fine</td>
<td>FW</td>
</tr>
<tr>
<td><strong>H:L ratio, %</strong></td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>45</td>
<td>41</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>42</td>
<td>46</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>Post-molt</td>
<td>47</td>
<td>40</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td><strong>Plasma Ca, mg/dL</strong></td>
<td>29.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>33.1</td>
<td>35.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>13.7*</td>
<td>12.2*</td>
<td>11.4*a</td>
<td>11.3*a</td>
</tr>
<tr>
<td>Post-molt</td>
<td>31.5</td>
<td>32.3</td>
<td>32.2</td>
<td>33.8</td>
</tr>
<tr>
<td><strong>Inorganic P, mg/dL</strong></td>
<td>1.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>1.42</td>
<td>1.48*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>1.06</td>
<td>1.01</td>
<td>0.96</td>
<td>1.07</td>
</tr>
<tr>
<td>Post-molt</td>
<td>1.32</td>
<td>1.36</td>
<td>1.33</td>
<td>1.41</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase, IU/L</strong></td>
<td>32.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium pre-molt</td>
<td>32.4</td>
<td>58.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>During molt</td>
<td>71.6*</td>
<td>65.5*</td>
<td>62.2*</td>
<td>66.0*</td>
</tr>
<tr>
<td>Post-molt</td>
<td>41.4</td>
<td>48.5*</td>
<td>45.0</td>
<td>50.4*</td>
</tr>
</tbody>
</table>

1Values are least squares means ± pooled SEM (n = 9).
2Calcium was supplied as a 50:50 mix of fine (0.14 mm mean diameter) and coarse (2.27 mm mean diameter) CaCO$_3$ or as an all-fine CaCO$_3$ mixed into a laying hen diet for a 1 wk pre-molt Ca treatment.
3Three molt diets were compared: feed withdrawal (FW), soybean hulls (SH), and wheat middlings (WM).
4P-values from main effect of Ca pre-molt treatment or molt diet.
\textsuperscript{a}Means within a row lacking a common superscript differ (P < 0.05).
\textsuperscript{b}Means within a row differ from baseline value (P < 0.05). P-value from Dunnett’s comparison.