Guar Meal Diets as an Alternative Approach to Inducing Molt and Improving *Salmonella* Enteritidis Resistance in Late-Phase Laying Hens

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**ABSTRACT** Induced molting of laying hens is a practice used by commercial egg producers to increase the productive lifetime of their flock. However, the conventional method of inducing molt, which involves removal of feed, water, or both as well as a reduction in photoperiod to less than a natural day has drawn criticism due to animal welfare and food safety concerns. The objective of this study was to explore the efficacy of diets containing high levels of guar meal (GM) in inducing molt and reducing susceptibility to *Salmonella* Enteritidis colonization in late-phase laying hens. Late-phase (68 wk old) Lohmann laying hens were either full-fed standard laying hen diets (nonmolted control), induced to molt by feed withdrawal, or full-fed standard laying hen diets containing 20% GM with or without 250 units/kg of mannanase Hemicell supplementation. On the fourth day of treatment, all hens were orally challenged with SE (1.65 × 10⁷ cfu). Hens were killed and evaluated for *Salmonella* colonization and differences in organ weights 5 d postinoculation. *Salmonella* Enteritidis present in crop, liver, ovary, and cecal contents were significantly reduced by feeding GM with enzyme supplementation compared with feed withdrawal hens. No significant differences were observed in reproductive tract weights of molted groups, although a difference in liver weight was detected. Results indicate that feeding diets containing 20% GM are as effective as complete feed withdrawal with respect to inducing molt with the added benefit of improved resistance to *Salmonella* Enteritidis colonization and translocation.

**Key words:** molt, laying hen, guar, *Salmonella*

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**INTRODUCTION**

Induced molting of late-phase laying hens is used by many commercial egg producers in the United States to stimulate multiple laying cycles and restore egg quality. Conventional methods of inducing molt involve fasting and in some cases removing water for a sufficient period of time to completely regress the tissues of the reproductive tract. However, using feed withdrawal to induce molt has drawn criticism due to concerns about the humane treatment of animals used for food production. Additionally, stress associated with long-term feed withdrawal impairs the immunological function of laying hens and increases the susceptibility of layers to *Salmonella* infection.

Several alternative methods to molting hens by feed withdrawal have evaluated the efficacy of full-feeding diets with a variety of mineral imbalances, including low-sodium diets (Berry and Brake, 1985), diets deficient in calcium (Martin et al., 1973), and diets high in zinc (Berry and Brake, 1985). Another approach to inducing molt in laying hens involves full-feeding grain and legume by-products rich in indigestible plant fibers such as alfalfa (Donalson et al., 2005), cottonseed meal (Davis et al., 2002), and guar meal (GM; Patel and McGinnis, 1981; Zimmermann et al., 1987). In addition to inducing molt, the use of wheat middlings (See et al., 2001) is also effective in reducing the incidence of *Salmonella* Enteritidis in eggs and internal organs.

Mannan oligosaccharides are believed to bind fimbriae present on the extracellular membrane of *Salmonella* cells, limiting their ability to bind and colonize intestinal epithelial cells (Fernandez et al., 2002). Guar (Cyamopsis tetragonoloba) meal, a by-product of guar gum processing, contains 18 to 20% gum residue (Bakshi et al., 1965; Nagpal et al., 1971). Guar gum is a linear polysaccharide of β-galactomannan consisting of a 1→4-linked β-D-mannopyranose backbone with branched 1→6-α-D-galactopyranose. Partially hydrolyzed guar gum (PHGG), produced by the hydrolysis of guar gum by β-endomannanase, is comprised of neutral polysaccharides consisting of a mannose backbone chain with single galactose side units occurring on approximately 2 out of every 3 mannose units.

Guar gum has been reported to elicit protective effects against the colonization of pathogenic bacteria within
the intestinal tract of rats (Noack et al., 1998). Ishihara et al. (2000) found that pullets and laying hens consuming PHGG had a decreased incidence of colonization by Salmonella Enteritidis in the organs and intestinal tract, with a concurrent increase in excretion of Salmonella Enteritidis into the feces. The same group also reported a decreased incidence of SE in egg components when PHGG was administered in the diet. These results suggest that the administration of guar gum or PHGG may prevent Salmonella Enteritidis colonization in pullets and laying hens.

The purpose of this experiment was to evaluate the use of GM, with and without enzyme treatment (Hemcell, ChemGen Corp., Gaithersburg, MD), as an alternative method of inducing molt and improving resistance to Salmonella infection in late-phase laying hens. Tissue regression rates of certain organs were measured in molted laying hens.

**MATERIALS AND METHODS**

**Experimental Design and Molting Procedure**

Forty-three Lohmann White late-phase laying hens (68 wk old) of similar BW (1,527 ± 114 g) were wing-banded and randomly allocated into 9 cages of a rearing battery housed in an environmentally controlled room. Eight cages had 5 hens each, and 1 cage had 3 hens, which served as a negative control group for the Salmonella Enteritidis colonization portion of this study (AUP 2003-0256, approved by the Institutional Agricultural Animal Care and Use Committee, Texas A&M University, College Station, TX). Hens were allowed to acclimate for 1 wk with free access to a typical corn-soy laying hen feed and water while on a 16L:8D lighting program. At the end of the acclimation period, hens were weighed individually. Four treatments, consisting of a feed withdrawal group (FW), full-fed groups combining standard laying hen feed with either 20% GM (20% GM) or 20% GM supplemented with 250,000 units/kg of β-mannanase (Hemcell; 20% + E), and a full-fed standard laying diet group (nonmolted control) were randomly assigned to 8 cages. Nutrient composition of GM (Table 1) was previously determined by Conner (2002) with amino acid analysis by Degussa-Huls Corporation (Applied Technology Chemical Group, Allenwood, NJ).

Beginning on the first day of treatment, the light program was changed to 8L:16D. On d 4 of treatment, all hens, with the exception of the 3 extra hens that served as a negative control group, were inoculated with 1 mL of Salmonella Enteritidis (1.65 × 10⁷ cfu/mL) by oral gavage. Eggs laid during the study were collected and recorded daily. All hens received free access to water.

**Salmonella Enteritidis Inocula**

A primary poultry isolate of Salmonella Enteritidis, obtained from the USDA National Veterinary Services Laboratory (phage type 13A), was selected for resistance to novobiocin (NO; No. n-1628, Sigma Chemical Co., St. Louis, MO) and to nalidixic acid (NA; No. n-4382, Sigma Chemical Co.) within our laboratory. Salmonella Enteritidis was grown according to the method of Lee and Falkow (1990), allowing for attainment of log-phase growth. Bacteria were washed 3 times in distilled water by centrifugation (3,000 × g) and spectrophotometrically quantified to a stock concentration of approximately 1 × 10⁹ cfu/mL in distilled water using a standard curve generated from comparison of multiple spread platings and optical densities. Bacteria were then diluted to a challenge concentration of 1.65 × 10⁷ cfu/mL as determined by multiple spread platings.

**Organ Weights and Salmonella Recovery**

Five days postinoculation (d 9 of treatment), all hens were euthanized by CO₂ asphyxiation, and the crop, liver, spleen, ovary, oviduct, and ceca of each bird were excised aseptically as described below. After clamping across the pre- and postcrop esophagi using surgical Carmalt forceps, the crop was sectioned aseptically with the lumen and contents intact and collected into individual Whirl-Pac bags. The whole liver, spleen, ovary, and oviduct (from infundibulum to shell gland) were excised aseptically and collected into individual Whirl-Pac bags separately and weighed. Twenty milliliters of tetrazolium-broth base (No. 0104-17-6, Difco Laboratories, De-

<table>
<thead>
<tr>
<th>Table 1. Composition of nonmolted control diet and molt-inducing diets containing 20% guar meal (GM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients (%)</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>GM³</td>
</tr>
<tr>
<td>Dehulled soybean meal</td>
</tr>
<tr>
<td>n-Met</td>
</tr>
<tr>
<td>L-Lys HCl</td>
</tr>
<tr>
<td>Fat (animal-vegetable blend)</td>
</tr>
<tr>
<td>Limestone</td>
</tr>
<tr>
<td>Monodicalcium phosphate</td>
</tr>
<tr>
<td>Salt</td>
</tr>
<tr>
<td>Trace minerals⁴</td>
</tr>
<tr>
<td>Vitamins⁵</td>
</tr>
</tbody>
</table>

¹Calculated analysis of all diets containing 20% GM was as follows: CP, 14.06%; ME, 2,893 kcal/kg; Ca, 2.00%; available P, 0.40%; Met, 0.32%; Lys, 0.68%; Thr, 0.42%; and Trp, 0.13%.
²Calculated analysis of nonmolted control diet was as follows: CP, 15.24%; ME, 3,000 kcal/kg; Ca, 0.80%; available P, 0.40%; Met, 0.14%; Lys, 0.72%; Thr, 0.54%; and Trp, 0.16%.
³The nutrient matrix used for GM was as follows: CP, 38.3%; ME, 2,033 kcal/kg; Ca, 0.16%; available P, 0.16%; Met, 0.45%; Lys, 1.64%; Arg, 4.90%; Thr, 1.04%; and Trp, 0.43%.
⁴Trace minerals premix added at this rate yields the following: 27.5 mg of S, 150 mg of Mn, 1.68 mg of Fe, 1.7 mg of Cu, 125.5 mg of Zn, 0.25 mg of Se, 1.05 mg of I, 0.84 mg of Molybdenum per kilogram of diet.
⁵Vitamin premix added at this rate yields the following: 11,023 IU of vitamin A, 46 IU of vitamin E, 3,850 IU of vitamin D₃, 1.47 mg of vitamin K, 2.94 mg of thiamin, 5.85 mg of riboflavin, 20.21 mg of pantothenic acid, 0.55 mg of biotin, 1.75 mg of folic acid, 477.67 mg of choline, 16.5 µg of vitamin B₁₂, 45.93 mg of niacin, and 7.17 mg of pyridoxine per kilogram of diet.
the presence of 

for 24 h at 37

samples were stored at 0

BGA plates were scored as 0 cfu. The remaining organ

incubated for another 24 h at 37

was individually streaked onto a NO-NA-BGA plate,

was aseptically sectioned, and approximately 0.5 g of cecal content was squeezed into a centri-

mL of tetrathionate broth base and vortexed for 30 s. One
cecum from each hen was aseptically excised, minced,
and collected into 50-mL conic centrifuge tubes with 30 

mixing was achieved by using a Laboratory Blender, Cincinnati, OH) for 60 s. 

cecum from each hen was aseptically excised, minced,
collected into 50-mL conic centrifuge tubes with 30 mL of tetrathionate broth base and vortexed for 30 s. The other cecum was aseptically sectioned, and approximately 0.5 g of cecal content was squeezed into a centrifuge tube containing 4.5 mL of Butterfield’s buffer solution. Each dilution (0.1 mL) was plated onto a brilliant green agar (BGA; No. 0285-01-5, Sigma Chemical Co.) plate containing NO 25 μg/mL and NA 20 μg/mL (NO-NA-BGA) to prohibit growth of Salmonella other than the antibiotic-resistant challenge isolate. Plates were incubated for 24 h at 37°C, and Salmonella Enteritidis number (cfu/g of cecal contents) was determined. Cecal contents in which Salmonella Enteritidis was not detected at the 10⁻⁴ dilution on BGA plates were scored as 0 cfu. The remaining organ samples were stored at 0°C for 108 h before incubation for 24 h at 37°C. After the enrichment phase, each sample was individually streaked onto a NO-NA-BGA plate, incubated for another 24 h at 37°C, and examined for the presence of Salmonella Enteritidis.

**Statistical Procedure**

Body weight reduction and absolute and relative weights of organs were subjected to ANOVA by the GLM procedure of the SAS System (SAS System for Windows, Release 8.1, SAS Institute Inc., Cary, NC) for a completely randomized design. Each hen served as an individual experimental unit. Feed consumption data were not collected. The presence of Salmonella Enteritidis in each organ was analyzed by Fisher’s exact test (Uittenbroek, 2000). The P-value for the same or a stronger association was used in Fisher’s test (Garson, 2004). The total Salmonella Enteritidis-positive numbers of organs from each group and from all hens were compared by Pearson’s χ² test. The number of Salmonella Enteritidis colony-forming units per gram of cecal content was first subjected to log transformation and then analyzed using the GLM procedure of the SAS System for a completely randomized design. Data from the 3 extra hens were not included when analyzing Salmonella Enteritidis presence and log colony-forming unit data. Significance was accepted at P ≤ 0.05.

**RESULTS**

**BW and Laying Activity**

Mean BW of each group was similar at the beginning of the trial but significantly diverged by the end of the study (Table 2). All induced-molt treatment groups completely ceased egg production by the sixth day of treatment, whereas nonmolted hens continued laying throughout the 9-d study.

**Absolute and Relative Organ Weight**

Liver weights of all groups were significantly different from each other. However, relative liver weight to BW for nonmolted hens and hens fed 20% GM + E were not different, and both were proportionally larger than the relative liver size of FW and 20% GM (Table 2). Nonmolted hens had significantly lower relative spleen weights than molt-induced hens, which were not different from each other. Nonmolted hens, which continued laying throughout the study, had significantly higher absolute and relative weights of ovary and oviduct than molted hens. Differences were not observed among the molt-induced groups.

**Salmonella Enteritidis Colonization and Organ Invasion**

Salmonella Enteritidis was not detected in the organ samples or cecal contents of nonchallenged negative control hens (Table 3). Compared with nonmolted control hens, all molt-induced hens had a higher incidence of Salmonella Enteritidis in cecum and oviduct with no
difference between molting methods. In liver samples, FW hens had higher incidences of SE than 20% GM + E and nonmolted control hens. The FW hens also had increased susceptibility to Salmonella Enteritidis in the crop and ovary than nonmolted hens and hens fed GM + E. More pronounceable differences between treatments were detected when the incidence of Salmonella Enteritidis colonization was summed across all organ samples. All molted hens had higher total Salmonella Enteritidis-positive numbers in the 6 tested organs than nonmolted hens. Among molt-induced groups, FW hens had a higher prevalence of Salmonella Enteritidis than hens fed 20% GM, which in turn were higher than hens fed 20% GM + E. With respect to colony-forming units of Salmonella Enteritidis in cecal contents, FW hens contained cecal populations more than 3 logs higher than those of either group of hens fed GM, which were 1.5 to 2 logs higher than that of nonmolted hens.

**DISCUSSION**

A reduction in BW of molted hens ranging from 25 to 35% is recommended to achieve optimal involution of the reproductive tract and subsequent postmolt egg production levels (Zimmermann et al., 1987; Carey and Brake, 1989). In this experiment, however, hens fed 20% GM + E experienced a BW loss of only 16%, whereas hens consuming 20% GM without enzyme supplementation showed a reduction in BW of 19%. Molted hens had losses in total weight of oviduct, ovary, and liver ranging from 92 to 107 g and losses due to regression of other tissues and organs than FW hens. Therefore, the BW loss occurring exclusive of the reproductive tract in FW hens may not be critical to molt induction. This finding suggests that the recommendation of a reduction in BW by 25 to 35% may not be suitable when a full-feeding alternative molting technique is used.

In the present study, hens molted by 20% GM diets had significantly reduced Salmonella Enteritidis colonization numbers in most organs, and the GM diet with enzyme supplementation had a stronger effect on Salmonella Enteritidis resistance than the GM diet without enzyme relative to fasted control groups. Evidence of the ability of GM to enhance Salmonella Enteritidis resistance of molted hens also was supported by the more than 3 log reduction of Salmonella Enteritidis colony-forming units of cecal contents relative to the effects of FW. Consistency between the occurrence of positive Salmonella Enteritidis organ samples and Salmonella Enteritidis colony-forming units per gram of cecal contents indicates that induced molting by full-fed GM diets improves resistance to Salmonella Enteritidis in molted hens.

With respect to days to 0 egg production, this study demonstrates that full-feeding late-phase laying hens with 20% GM with and without β-mannanase (Hemicell) appears to be as effective in inducing molt as the conventional FW method and is potentially more desirable with respect to animal welfare concerns due to a presumed decrease in stress associated with BW loss. Additionally, laying hens induced to molt by GM feeding exhibit improved resistance to Salmonella Enteritidis colonization when compared with hens molted by complete feed withdrawal. Furthermore, supplementation of β-mannanase (Hemicell) to diets containing high levels of GM appears to enhance resistance to Salmonella Enteritidis colonization in molted laying hens.

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**REFERENCES**


**Table 3. Colonization and invasion of Salmonella Enteritidis into organs and Salmonella Enteritidis colony-forming units in cecal contents of laying hens induced to molt by various methods**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hens (n)</th>
<th>Crop</th>
<th>Liver</th>
<th>Spleen</th>
<th>Ovary</th>
<th>Oviduct</th>
<th>Cecal</th>
<th>All organs of cecal content</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW control</td>
<td>10</td>
<td>7/10^a</td>
<td>9/10^a</td>
<td>3/10^b</td>
<td>7/10^a</td>
<td>8/10^a</td>
<td>10/10^a</td>
<td>44/60^e</td>
</tr>
<tr>
<td>20% GM</td>
<td>10</td>
<td>0/10^a</td>
<td>6/10^a</td>
<td>5/10^a</td>
<td>3/10^b</td>
<td>5/10^a</td>
<td>9/10^a</td>
<td>28/60^d</td>
</tr>
<tr>
<td>20% GM + E</td>
<td>10</td>
<td>0/10^e</td>
<td>1/10^a</td>
<td>1/10^b</td>
<td>1/10^e</td>
<td>5/10^a</td>
<td>9/10^a</td>
<td>17/60^a</td>
</tr>
<tr>
<td>Nonmolt</td>
<td>10</td>
<td>2/10^a</td>
<td>0/10^b</td>
<td>0/10^b</td>
<td>0/10^b</td>
<td>0/10^b</td>
<td>2/10^b</td>
<td>4/60^c</td>
</tr>
<tr>
<td>Nonchallenged</td>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/18</td>
</tr>
</tbody>
</table>

**Log Salmonella Enteritidis colony-forming units (cfu/g of cecal content).**

**Means in the same column lacking a common superscript were significantly different (P ≤ 0.05).**

**FW = feed withdrawal; GM = guar meal; E = 250,000 units/kg of β-mannanase.**

**Data from nonchallenged hens were not included in the Salmonella Enteritidis colony-forming unit analysis.**