Melengestrol Acetate as an Effective Alternative to Induce a Decline in Egg Production and Reversible Regression of the Reproductive Tract in Laying Hens. II. Effects on Postmolt Egg Quality

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ABSTRACT Induced molting increases egg quality and egg production and extends the productive life of hens. Molting is accomplished by feed withdrawal, which has received criticism, and alternatives described thus far result in poor postmolt performance. Melengestrol acetate at a dosage of 4 or 8 mg/d, in a balanced diet, leads to reversible regression of the reproductive tract. However, this alternative must also increase egg quality after rest to be considered an adequate method by the industry. Hy-Line W-36 (n = 497) laying hens were assigned randomly to a diet containing 0 mg of melengestrol acetate (MGA; control) throughout the experiment or 4 or 8 mg of MGA/d for 2, 4, or 6 wk. Upon reaching 50 and 70% lay, after MGA removal, eggs were collected for measurements of egg quality, including Haugh units (i.e., internal egg quality), shell thickness, and breaking strength (i.e., external egg quality). Haugh units were greater ($P < 0.05$) for eggs laid by hens molted with a diet containing 8 mg of MGA for all durations compared with controls. Shell thickness was greater ($P < 0.05$) when hens were treated with 4 mg of MGA for 6 wk and 8 mg of MGA for 4 and 6 wk compared with control. Egg breaking strength was greater ($P < 0.05$) than controls for all hens fed MGA, regardless of dosage or duration of feeding. A subset of hens was fed 8 mg of MGA per hen/d for 2 wk, and eggs were collected for 3 wk. Seven days after MGA was removed from the diet, the amount of MGA in the yolk was below the level of detection of the assay, and the concentration found in the eggs at all time points was 3 orders of magnitude below the Food and Drug Administration's tolerance for MGA in edible tissue. When used as an alterative method to induce a rest, MGA leads to an increase in the internal and external egg quality of hens compared with nonmolted hens.

(*Key words*: chicken, molting, melengestrol acetate, well-being)

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INTRODUCTION

Inducing hens to molt results in increased production and egg quality and extends the productive life of a hen. Traditionally this is done by feed withdrawal, and as a result traditional molting practices do not adequately address hen well-being. Currently there are several proposed alternative methods to induce molting, but these alternatives also do not adequately address concerns of hen well-being. Many of the current alternatives are centered on the idea of altering a hen's daily nutrient intake. Examples of these alternatives include feeding low nutrient density diets (i.e., wheat middlings) and diets with altered macro minerals (i.e., low calcium, low sodium, or high zinc diets). These alternatives attempt to address hen well-being by maintaining hens on some form of feedstuff during molt. However, these alternatives have been found to increase instances of hen paralysis, kidney and adrenal damage, and hen dehydration (Siegel, 1961; Lumijarvi et al., 1966; Douglas et al., 1972; Berry, 2003)

Traditional molting thus far is not only the most consistent and repeatable method, but it creates the greatest improvements in postmolt performance. Current alternative methods are not predictable or repeatable among experiments (Berry, 2003). Most alternatives do not increase egg quality above the designated control following a molt, as is observed with low calcium and high zinc and wheat middling alternatives (Nevalaine, 1969; Douglas et al., 1972; Shippee et al., 1979; Biggs et al., 2003, 2004).

Traditional molting practices result in a rapid decrease in egg production and cessation within 1 wk, but total time spent molting (i.e., cessation of lay, through peak

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Abbreviation Key: LH = luteinizing hormone; MGA = melengestrol acetate.

production) is approximately 9 wk, which means that hens are at a limited production level for a rather lengthy period. Hens that are maintained on a balanced layer diet during molting are not expected to deplete body stores, which would result in a shorter recovery period from the onset of molting to peak postmolt production. The key component to increase postmolt performance is reversible regression of the reproductive tract, especially the epithelial cells that line the oviduct, not an overall reduction in hen body weight (Brake and Thaxton, 1979). We have previously demonstrated that feeding 4 or 8 mg of melengestrol acetate (MGA) per hen/d in a balanced layer diet results in reversible regression of the reproductive tract (Koch et al., 2005) and does not result in inadequate hen well-being (Koch et al., 2004). Therefore, the objective of the current experiment was to determine if egg quality is improved following an MGA induced molt and if the time required to increase egg quality is shorter than that required for traditional molt.

MATERIALS AND METHODS

Hy-Line W-36 laying hens (n = 497) at 65 wk of age were used for the experiment. All hens were housed 3 per cage with 186 cm^2 of floor space per bird and exposed to 18 h of light per day. Birds were fed a balanced layer diet that was formulated to meet National Research Council requirements (NRC, 1994) and provided ad libitum access to water for 7 wk before the start of the experiment. All procedures involving animals were approved by the West Virginia University Animal Care and Use Committee (02-1203).

Hens were assigned randomly to an incomplete factorial design in which the control group (0 mg of MGA; n = 65) was fed a balanced layer diet throughout, and the treated groups received 4 (n = 72/duration) or 8 (n = 72/duration) mg of MGA (International Nutrition, Omaha, NE) per d in a balanced diet (Koch et al., 2005) for 2, 4, or 6 wk and then were returned to a balanced layer diet without MGA. Eggs were collected prior to feeding MGA. Eggs were also collected for 4 consecutive days when treatment groups reached 50 and 70% production after MGA was removed from the diet (i.e., postmolt) for assessment of egg quality. Upon collection, the eggs from each day were separated into 2 groups. One group of eggs was stored at 4°C for no more than 3 wk until breaking strength was tested, and the other group was tested immediately for albumen height, egg weight, and shell thickness.

Internal egg quality was determined by Haugh units {Haugh units = $100 \times \log[\text{albumen height} + 7.57 - (1.7 \times \text{egg weight}^{.037})]$}, which includes albumen height and egg weight. Egg weight (g) and albumen height (mm) were determined using a QCBI digital balance (TSS, York, UK) and QCH albumen height gauge (TSS), respectively. External egg quality was measured by shell thickness (μm; QCT shell thickness micrometers, TSS) and breaking strength (g; QC-SPA shell strength analyzer, TSS).

An additional subset of hens (n = 12) that were 80 wk of age were housed individually and kept separate from those in other portion of the experiment and fed 8 mg of MGA/d for 2 wk, and eggs were collected starting with the last egg that was laid while birds were fed a diet containing MGA and every day following for 3 wk. Eggs laid by hens fed the same ration without incorporation of MGA were used as controls. The eggs that were collected were separated into the albumen and yolk, which was homogenized and stored at –80°C until assayed. Extraction of the yolk and albumen was modified from the procedure described by the manufacturer for extraction of MGA from muscle or adipose tissue. One gram of yolk or albumen was added to 15 mL of petroleum ether, vortexed, and placed in a 40°C shaking water bath overnight. The phases were separated by freezing the extracted yolk or albumen at –20°C for 1 h and then centrifuging for 15 min at $2{,}000 \times g$ at –15°C. The petroleum ether was then decanted into a new vial and evaporated to dryness in a 60°C water bath. The residue was redissolved in 2 mL of methanol and defatted at –80°C for 45 min. The precipitated fats were pelleted at $2{,}000 \times g$ for 5 min at –15°C. The supernatant was decanted, and distilled H_2O was added to obtain a final ratio of 40:60 (methanol:H_2O). The MGA content in the resultant extract was determined utilizing a commercially available enzyme immunoassay (Lot No. 03303, Ridascreen R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions. Extraction efficiency, as determined by the recovery of MGA added to control yolk, was 62.3%, which was similar to that indicated by the manufacturer for bovine perirenal fat (approximately 65%). The assay cross-reactivity with progesterone is less than 0.003%. The concentration of progesterone was determined on the same samples, using radioimmunoassay (Sheffel et al., 1982)

Statistical Analysis

Data for Haugh units, shell thickness, and breaking strength were analyzed using the GLM procedure of SAS (SAS Inst., Cary, NC). Means were separated using Duncan's multiple comparison test. Data were not different for eggs collected at 50 and 70% lay, and so data were pooled. The model included the amount of MGA and the duration of feeding. $P < 0.05$ was considered significant.

RESULTS

There was no effect of the duration of MGA feeding on internal egg quality so the data were pooled within an MGA treatment. Postmolt internal egg quality (i.e., Haugh units) was the greatest when hens received 8 mg of MGA/d, regardless of the duration of feeding (81.06 ± 0.70). The internal egg quality in the group receiving 4 mg of MGA per hen/d for all durations was not greater than those of the controls (Figure 1).

The egg weight observed in the control group was similar to the postmolt egg weight of those groups receiv-

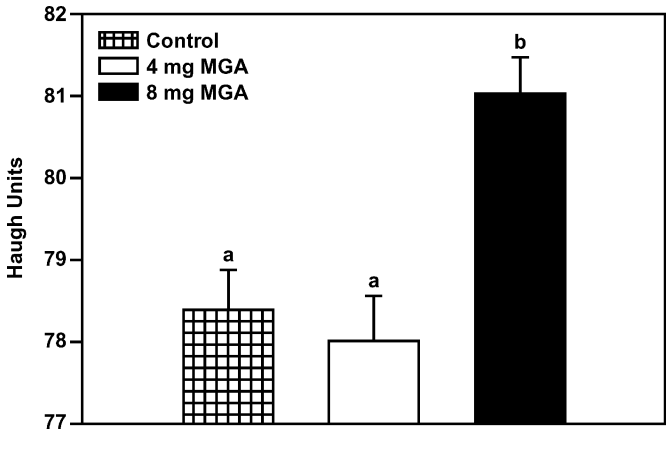

Figure 1. Internal egg quality as measured by Haugh units (%) for egg laid by hens fed 0, 4, or 8 mg of melengestrol acetate (MGA) per hen per day, regardless of the duration of MGA feeding. [a,b]Means ± SEM with different letters are significantly different ($P < 0.05$).

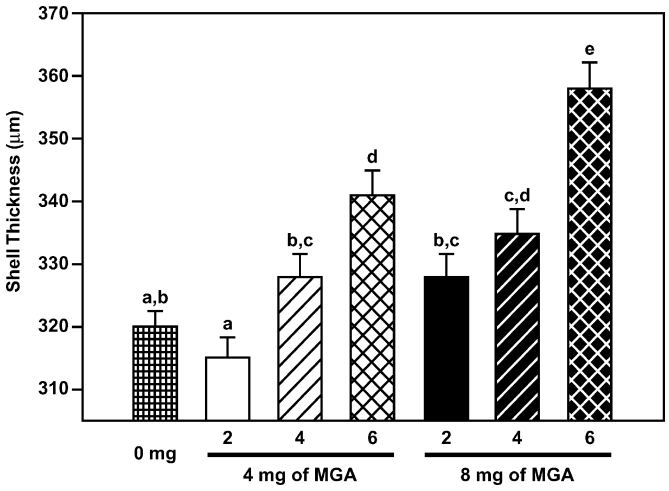

Figure 2. External egg quality as measured by shell thickness (mm) for eggs laid by hens fed either 0 mg of melengestrol acetate (MGA) throughout the experiment or 4 or 8 mg of MGA per hen/d for 2, 4, or 6 wk. [a–e]Means ± SEM with different letters are significantly different ($P < 0.05$).

ing either 4 mg of MGA for all durations or 8 mg of MGA for 2 of 4 weeks (Table 1). However those receiving 8 mg of MGA for 6 wk had an increase in postmolt egg weight (Table 1).

External egg quality as measured by shell thickness increased as the duration of MGA feeding increased in the groups receiving 4 or 8 mg of MGA. Shell thickness was greater when 4 mg of MGA was fed for 6 wk than the shell thickness of the control group (Figure 2). Shell thickness was greater in the groups receiving 8 mg of MGA for 4 or 6 than those receiving 0 mg of MGA (Figure 2).

Breaking strength was greater in the groups receiving 4 or 8 mg MGA for all durations compared with those receiving 0 mg of MGA. Those receiving 4 mg of MGA for 4 or 6 wk had a greater breaking strength than those receiving 4 mg for 2 wk (Figure 3). However, the breaking strength observed in those groups receiving 4 mg of MGA for 4 or 6 wk was not different from the breaking strength of the groups receiving 8 mg of MGA for 2, 4, or 6 wk (Figure 3).

Egg production rapidly decreased after adding MGA to the diet and continued to decline until hens were returned to a balanced layer diet without MGA. Those receiving 4 mg of MGA reached a low production of 30.3%, whereas those receiving 8 mg of MGA had a decline in production to 9.0% (Table 2). Upon removal of MGA from the diet, egg production in all groups rapidly increased and reached the level of the controls (Table 2).

The concentration of MGA found in the yolk after MGA removal was approximately 0.45 ppb (Figure 4). The concentration dramatically decreased until the concentration of MGA was below the level of detection of the assay (0.10 ppb) at d 7 (Figure 4). The MGA was not found at any concentration in the albumen (data not shown). A graph of the concentration of MGA relative to the FDA tolerance level for MGA in edible tissue (25 ppb, Department of Health and Human Services, Food and Drug

Table 1. Egg weight for the different dosages of melengestrol acetate (MGA) fed for 2, 4, or 6 wk

Amount of MGA and duration of feeding	Egg weight (g) average
0 mg	65.2[ab]
4 mg of MGA	
2 wk	64.9[a]
4 wk	64.7[a]
6 wk	65.4[ab]
8 mg of MGA	
2 wk	65.9[bc]
4 wk	65.7[ab]
6 wk	66.8[c]

[a-c]Means ± SEM with different superscripts differ ($P < 0.05$).

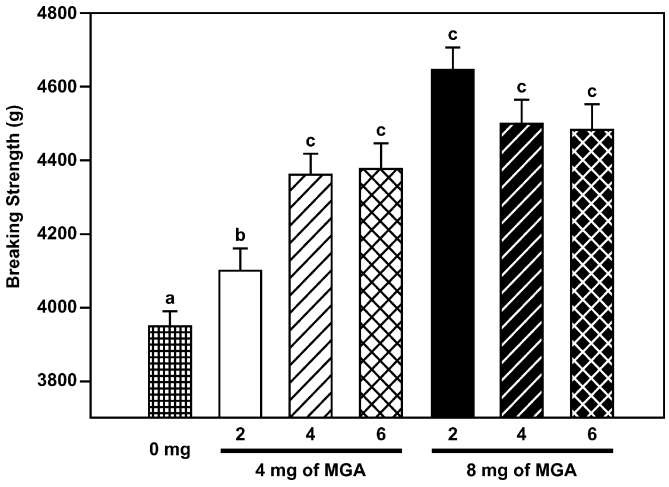

Figure 3. External egg quality as measured by breaking strength (g) for egg laid by hens fed 0 mg of melengestrol acetate (MGA) throughout the experiment or 4 or 8 mg of MGA per hen/d for 2, 4, or 6 wk. [a–c]Means ± SEM with different letters are significantly different ($P < 0.05$).

Table 2. Egg production for all treatment groups during a 12-wk experiment

Treatment (mg of MGA[1] and duration of feeding)	Production (%)											
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12
0 mg	81.6	75.0	76.2	74.6	85.7	81.5	76.6	77.5	84.7	82.3	72.6	83.1
4 mg of MGA												
2 wk	85.8	54.2	47.2	55.6	61.8	72.2	77.8	79.2	71.5	72.2	88.9	77.1
4 wk	83.3	60.4	46.5	27.8	39.6	57.6	63.9	78.5	74.3	75.7	84.0	76.4
6 wk	83.0	60.4	45.4	31.0	37.3	30.3	35.9	50.7	71.8	74.6	74.0	82.2
8 mg of MGA												
2 wk	79.0	32.0	20.7	33.6	49.3	62.5	69.3	74.3	85.0	71.4	78.6	87.2
4 wk	84.2	25.7	18.8	16.0	18.8	33.3	48.6	54.3	65.7	70.8	80.7	78.6
6 wk	90.2	18.8	12.5	9.7	13.2	9.0	13.2	21.8	56.4	71.8	69.8	83.8

[1]MGA = melengestrol

Administration, 1994) and the endogenous progesterone concentration (approximately 100 ppb) demonstrates that yolk MGA concentration is 3 orders of magnitude below the Food and Drug Administration's tolerance level and 6 orders of magnitude below the endogenous progesterone concentration (Figure 5).

DISCUSSION

Inducing hens to molt by incorporating MGA into a balanced layer diet at a level of 4 or 8 mg per hen/d for varying durations of feeding resulted in an improvement in egg quality. Breaking strength, a measure of external egg quality, was improved with all MGA amounts and durations. The greatest improvement was observed in the eggs laid by hens that received 8 mg of MGA for 2, 4, or 6 wk. This improvement was similar to the improvement observed from those eggs laid by hens receiving 4 mg of MGA but only for 4 or 6 wk. The groups receiving 4 mg of MGA for 2 wk exhibited an improvement in breaking strength intermediate between the controls and the other groups. Shell thickness, another measure of external egg quality increased as the duration of MGA feeding increased in both treatment groups. The 2 measures used in this experiment to assess external egg quality did not follow similar patterns, as one might expect. However, it has been found that only 56% of variation in breaking strength can be attributed to shell thickness and other components, including egg size, egg structure, and curvatures, play an important role in determining breaking strength (Richards and Swanson, 1965; Rodriguez-Navarro et al., 2002). The greatest improvement in egg quality was observed in the hens receiving 4 or 8 mg of MGA for 6 wk. Melengestrol acetate at 8 mg/hen per day at all durations resulted in greatest internal egg quality (as measured by Haugh units).

Previously, experiments have demonstrated that hens can be induced to molt by the administration of hormones (Gabuten and Shaffner, 1954; Shaffner, 1954; Adams, 1955; Adams, 1956; Dickerman and Bahr, 1989), but the number of experiments done to assess postmolt egg quality are limited. However, the key to increasing postmolt performance is inducing a molt that leads to reversible regres-

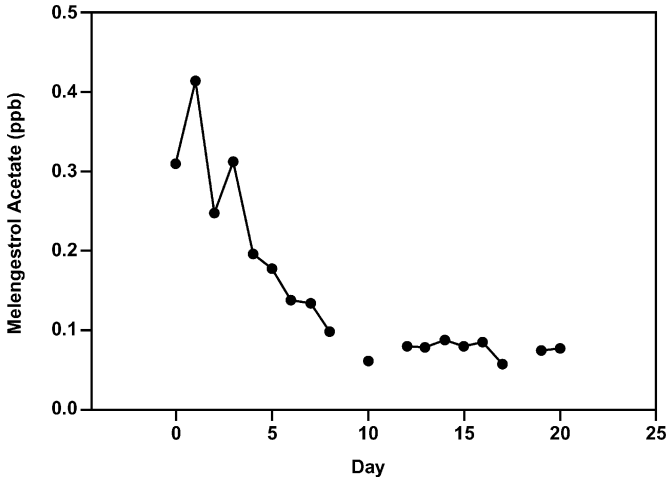

Figure 4. Yolk melengestrol acetate (MGA) concentration (ppb) in the days following MGA removal from the diet. Day 0 eggs are representative of the last egg laid while hens were on the last day of a 14-d MGA treatment.

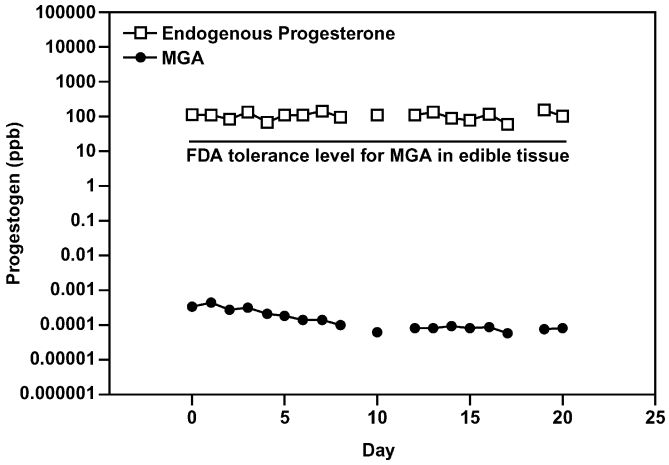

Figure 5. Yolk melengestrol acetate (MGA) and endogenous progesterone concentration in the days following removal of MGA from the diet, graphed relative to the Food and Drug Administration's (FDA) tolerance for MGA in edible tissue (Code of Federal Regulations 21CFR 556.380). Day 0 eggs are representative of the last egg laid while hens were on the last day of a 14-d MGA treatment. The concentration of yolk MGA for those eggs collected on d 0 is 3 orders of magnitude below the FDA's tolerance level and 6 orders of magnitude below the endogenous progesterone concentration.

sion of the reproductive tract (Brake, 1993). The alternative method that is the most successful at causing reversible regression that results in an increased postmolt performance and addresses hen well-being will be the most desired alternative method.

Current alternative methods to a molt induced by feed withdrawal are all associated with altering the daily nutrients needed to maintain egg production and required for daily hen maintenance. These alternatives include dietary mineral alterations, such as low calcium, low sodium, or high zinc diets (Nevalaine, 1969; Douglas et al., 1972; Nesbeth et al., 1976a,b; Shippee et al., 1979; Naber et al., 1984; Berry and Brake, 1987). Another main alternative that has recently been looked into is feeding low nutrient density diets, such as wheat middling (Biggs et al., 2003, 2005). However, all of these alternatives are potentially less desirable to the industry because they lack the ability to increase postmolt performance or do not adequately address concerns of hen well-being. If an alternative method is going to replace feed withdrawal, then it must result in better postmolt performance and adequately address hen well-being concerns that are not addressed by traditional feed withdrawal. Simply providing a feedstuff that has not been demonstrated to improve hen well-being over feed withdrawal will not suffice.

These alternative methods are able to disrupt the physiology of the lay cycle and in return disrupt lay. Calcium is important for several components of egg production. Calcium is needed for the gonadotropin-releasing hormone stimulation of the luteinizing hormone (LH) surge and is required for LH stimulated progesterone production (Brake, 1993). Besides playing a role in reproduction, calcium functions as a major component in hard shell formation. Sodium is an important mineral needed to maintain the water balance in a body, and inadequate supplementation of sodium in a corn- and soybean-based layer diet can have profound effects on hen health. Feeding a high zinc diet results in extreme hen weight loss as a result of reduced feed intake. High concentrations of zinc have been found to limit the formation of cyclic adenosine monophosphate associated with the LH receptor, which decreases the production of progesterone (Brake, 1993).

When subjecting food source animals to steroids or other drug treatments, there is always concern about the level that can be detected in edible tissue and the amount of time after treatment that it can be detected. To address these concerns the concentration of MGA in the eggs was determined. The amount of MGA found in the yolk was well below (i.e., 3 orders of magnitude) the Food and Drug Administration's tolerance level for MGA in edible tissue and represented a miniscule amount of the total progestin in eggs.

The egg quality data presented in this paper compare the postmolt performance of a control group that was not molted (i.e., represent the before molt egg quality) and those molted with MGA. Comparison of traditional molting to a MGA induced molt will be more beneficial. However, the fact that feeding MGA improves internal and external egg quality is promising. Another component to an MGA-induced molt is the time required to reach 70% postmolt production. This is likely because hens are maintained on a balanced layer diet so the resting phase (i.e., the period after molting when hens are maintained on restricted protein diet to increase body conditioning) is shortened. Further testing is needed to perfect this method and make it more industry friendly. Changing the duration of artificial lighting has been shown to decrease the duration between the molting treatment and production, which has been found to be a key factor increasing egg quality postmolt (Hurwitz et al., 1995). If lighting or some other component can increase the effectiveness of an MGA induced molt then, the amount of MGA or the duration of feeding may be reduced.

In conclusion, incorporating MGA into a balanced layer diet induces molting in hens while they are maintained on a balanced layer diet. Maintaining hens on a balanced layer diet throughout molting prevents hen weight loss and decreases a hens motivation to obtain feed (Koch et al., 2005; Koch et al., 2004), which addresses concerns of hen well-being that have arisen regarding feed withdrawal and other methods associated with nutrient manipulation. Induction of molt by MGA allows for a quick recovery to peak production and increases internal and external egg quality.

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