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*Research Article*

# **Effects of Hen Age and Force Molting Programs on Body Weight Loss and Heterophil: Lymphocyte Ratio during Molt Period in Laying Hens**

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## **Abstract**

The aim of this study was to determine the effects of hen age and force molting programs on body weight loss, egg production cease and heterophil:lymphocyte ratio during molt period. The experiment was conducted using 320 Hy-Line W36 hens randomly assigned to experimental groups (five replicates of 8 hens for each treatment). The experimental design of the study was a 2 × 4 factorial arrangement of a completely randomized design. Eight treatments were compared in a 2 x 4 factorial arrangement with 2 age treatment (63 and 75 week of age) and 4 force molting treatments [feed withdrawal (FW), 100% alfalfa (A), 50% alfalfa and 50%oat (AO), and 100% oat (O)]. The hen age had no significant effect on BW loss, cease egg production and H: L ratio during molt period. The FW had significantly ( $P<0.01$ ) higher BW loss than AO and O treatments and it was similar to A treatment. Egg production for all treatments ceased completely by day 6. H: L ratio were significantly higher for FW treatment when compared with nonfeed withdrawal treatments. The results indicated that alfalfa or oat appears to be alternatives to FW methods for force molting.

**Keywords:** Laying hens, Force molting, Alfalfa, Oat, Heterophil:Lymphocyte ratio.

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## **Introduction**

Induced molting is practiced to give the flock a rest at the end of a period of egg production and to extend the productive life of their hens (North and Bell 1990). There are several force molting methods. Feed withdrawal has been the most popular due easy of application, economic benefits and agreeable postmolt performance (Bell 2003). However, these programs raise concern

about animal welfare because it is thought that they may be harmful to hens (Webster 2003). For this reason, researchers have examined alternative molting methods. These methods included high zinc (Sarica et al 1996; Yilmaz and Sahan 2003), low sodium concentrations (Berry and Brake 1985), wheat middlings (Biggs et al 2003), barley (Onbasilar and Erol 2007), cottonseed meals (Davis et al 2002), jojoba meal (Vermaut et al 1997), alfalfa (Donalson et al 2005; Landers

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et al 2005; Aygun and Olgun 2010; Aygun, 2013), and oat (Kocak et al 1980; Yetisir et al 1985; Tona et al 2002; Aygun and Yetisir 2009), which have been successfully used for induced molting. The feedstuff which used for alternative induced molting has usually insoluble plant fiber and low energy (alfalfa, cottonseed, grape pomace, and wheat middlings).

To the best of our knowledge, no previous studies have been carried out specifically on the effects of hen age and nonfeed withdrawal methods on body weight loss and heterophil: lymphocyte ratio during molt period in laying hens. There is only one study that investigated the effects of hen age on post molt performance. This is the first study that the effects of nonfeed withdrawal methods on some parameters during molting period. The aim of this study was to determine the effects of hen age and force molting programs on body weight loss, cease egg production and heterophil: lymphocyte ratio during molt period.

### Materials and Methods

A total of 320 Hy-Line W36 laying hens (63 and 75 week of age) were obtained from The Research and Application Farm at the Faculty of Agriculture at Selcuk University (Konya, Turkey). Hens were placed four hens per cage (500 cm<sup>2</sup>/hen), and 2 wk were allowed for acclimation. During this time the hens were fed a layer diet (Table 1) and the photoperiod was 16L:8D. After the acclimation, the hens were randomly assigned to experimental groups (five replicates of 8 hens for each treatment). The experimental design of the study was a 2 × 4 factorial arrangement of a completely randomized design. Eight treatments were compared in a 2 × 4 factorial arrangement with 2 age treatment (63 and 75 week of age) and 4 force molting treatments during 10 days [feed withdrawal (FW), 100% alfalfa

(A), 50% alfalfa and 50%oat (AO), and 100% oat (O)]. Thus 320 hens used in 8 treatments (2 age × 4 molting methods; 5 replicated) with 40 hens used in each. Alfalfa is very high in crude fiber (20-24%), has a moderate protein level (17-20 %) and has a low metabolizable energy (ME) value (1200-1600 kcal/kg) (NRC 1994) . The oat has got insoluble high fiber (10-11%) and moderate energy (2500 kcal/kg) (NRC, 1994), The respective diet and water were allowed ad-libitum, and hens were placed on an artificial lighting program of 8L:16D during the 10 d molt period (Donalson et al 2005; Petek et al 2008; Aygun and Olgun 2010; Aygun, 2013).

Egg production and mortality were recorded daily throughout during molt period. Hens were weighed on day 0, day 5 and day 10 to calculate body weight (BW) loss during molt period. Blood samples were taken from the brachial vein of one hen per replicate on day 0 and day 10 (end of the molt). Each hen sampled on day 0 was leg-banded after blood was drawn, and the same hen from each replicate group was sampled on day 10. The smears were stained with May-Grunwald-Giemsa stain. The total leukocyte count included heterophils, lymphocytes, monocytes, basophils and eosinophils. A total of 100 cells were counted for each ratio. The heterophil:lymphocyte (H: L) ratio was calculated by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel 1983).

### Statistical Analysis

Body weight loss, heterophil:lymphocyte ratio and cease egg production were analyzed using the general linear model (GLM). The least significant difference (LSD) test was applied to detect statistically significant differences between groups. All analyses were carried out using Minitab Version 14 (Minitab Inc., State College, PA, USA).

**Table 1: Composition of the Diet**

| Item (% , unless noted)<br>Ingredient  | Amount |
|--|--------|
| Corn, yellow   | 62.41  |
| Soybean meal, 47%  | 18.63  |
| Sunflower meal, 35%  | 2.50   |
| Vegetable oil  | 2.80   |
| Limestone  | 11.50  |
| Dicalcium phosphate  | 1.30   |
| Salt   | 0.35   |
| Vitamin premix <sup>1</sup>  | 0.15   |
| Mineral premix <sup>2</sup>  | 0.10   |
| Lysine   | 0.08   |
| DL-Methionine  | 0.18   |
| Total  | 100    |
| Calculated value   |        |
| CP   | 15.00  |
| ME (kcal/kg)   | 2800.0 |
| Ca   | 4.30   |
| Available P  | 0.34   |
| Lysine   | 0.80   |
| Methionine   | 0.38   |
| Methionine + cystine   | 0.67   |
| Threonine  | 0.56   |
| Tryptophan   | 0.17   |
| <sup>1</sup> Vitamin premix supplied per kilogram of diet: Vitamin A, 8 000 IU; vitamin D3, 2 200 IU; vitamin E, 13 IU; vitamin K3, 3 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 3 mg; vitamin C, 50 mg; calcium D-pantothenate, 7 mg; nicotine acid, 17 mg; D-biotin, 0.3 mg; folic acid, 0.67 mg; vitamin B12, 10 mg. |        |
| <sup>2</sup> Mineral premix supplied per kilogram of diet: Copper, 5 mg; iron, 60 mg; manganese, 100 mg; zinc, 60 mg; selenium, 0.15 mg; cobalt, 0.50 mg; choline, 125 mg.   |        |

## Results and Discussion

### Body Weight (BW) Loss

The effects of hen age and force molting programs on BW loss are shown in Table 2. Neither age nor molting programs had a significant effect on BW loss at 5 day of molt period. The hen age had no significant effect on BW loss at the end of the molt period (day 10). However, there were significant

( $p < 0.01$ ) differences between force molting programs for BW lost at the end of the molt period (day 10). The FW treatment (28.68%) significantly ( $p < 0.01$ ) higher BW loss than AO (19.70%) and O (19.77%) treatments. But no significant differences were observed between FW (28.68%) and A (24.49%) treatments. Similar results were found by Donalson et al (2005) who reported that hens fed 100% alfalfa were not significantly different from FW hens.

**Table 2: Effects of Hen Age and Force Molting Programs on BW Loss during Molt Period**

|                  |    | Body weight loss (%) |                     |
|------------------|----|----------------------|---------------------|
|                  |    | Day 5                | Day 10              |
| Age (week)       | 63 | 20.04                | 23.84               |
|                  | 75 | 18.98                | 22.49               |
| <i>SEM</i>       |    | 0.43                 | 0.42                |
| <i>P- value</i>  |    | >0.05                | >0.05               |
| Molting Programs | FW | 20.91                | 28.68 <sup>a</sup>  |
|                  | A  | 19.65                | 24.49 <sup>ab</sup> |
|                  | AO | 18.65                | 19.70 <sup>b</sup>  |
|                  | O  | 18.83                | 19.77 <sup>b</sup>  |
| <i>SEM</i>       |    | 0.61                 | 0.60                |
| <i>P- value</i>  |    | >0.05                | <0.01               |
| 63 week          | FW | 21.77                | 30.75               |
|                  | A  | 19.89                | 24.93               |
|                  | AO | 19.73                | 20.07               |
|                  | O  | 18.75                | 19.59               |
| 75 week          | FW | 20.05                | 26.62               |
|                  | A  | 19.41                | 24.05               |
|                  | AO | 17.57                | 19.34               |
|                  | O  | 18.91                | 19.94               |
| <i>SEM</i>       |    | 0.86                 | 0.85                |
| <i>P- value</i>  |    | >0.05                | >0.05               |

FW: Feed withdrawal, A: Alfaalfa, AO: 50% Alfalfa + 50% Oat, O: Oat

SEM: Standard error of the mean

There were no significant differences between A (24.49%) and AO (19.70%) and O (19.77%) programs for BW loss at day 10 of the molt period. This agrees with the findings of Khajali et al (2008), Petek and Alpaly (2008), Aygun and Olgun (2010), who reported that no significant differences in BW loss were observed among nonfeed withdrawal programs. BW losses in all treatments are in agreement with the report of Ruszler (1998) who state that for successful molting the BW loss should be between 15-40% during molting period. BW loses value of AO (19.70%) and O (19.77%) is similar to the values reported by Petek and Alpaly (2008), Aygun and Olgun (2010), and Aygun (2013).

### Egg Production Cease

There were no significantly differences in cease egg production between 63 week (5.85 day) and 73 week (5.85 day) age hens (Table 3). No significant differences in cease egg production were found among FW (5.70 day), A (6.00 day), AO (5.80 day), and O (5.90 day) treatments. This agrees with the findings of Donalson et al (2005) who reported that no significant differences in cease egg production between hens molted by alfalfa and feed withdrawal treatment. Gutierrez et al (2008) and Willis et al (2009), who stated that hens molted with nonfeed withdrawal ceased production on day 6. North and Bell (1990) stated that short resting periods can get a flock back to its peak of production in a month from the initiation of the molt.

**Table 3: Effects of Hen Age and Force Molting Programs on Cease Egg Production during Molt Period**

|                  |    | Cease egg production (day) |
|------------------|----|----------------------------|
| Age (week)       | 63 | 5.85                       |
|                  | 75 | 5.85                       |
| <i>SEM</i>       |    | <b>0.17</b>                |
| <i>P- value</i>  |    | <b>&gt;0.05</b>            |
| Molting Programs | FW | 5.70                       |
|                  | A  | 6.00                       |
|                  | AO | 5.80                       |
|                  | O  | 5.90                       |
| <i>SEM</i>       |    | <b>0.23</b>                |
| <i>P- value</i>  |    | <b>&gt;0.05</b>            |
| 63 week          | FW | 5.60                       |
|                  | A  | 6.00                       |
|                  | AO | 5.60                       |
|                  | O  | 6.20                       |
| 75 week          | FW | 5.80                       |
|                  | A  | 6.00                       |
|                  | AO | 6.00                       |
|                  | O  | 5.60                       |
| <i>SEM</i>       |    | <b>0.33</b>                |
| <i>P- value</i>  |    | <b>&gt;0.05</b>            |

FW: Feed withdrawal, A: Alfaalfa, AO: 50% Alfalfa + 50% Oat, O: Oat  
SEM: Standard error of the mean

#### **Heterophil: Lymphocyte (H: L) Ratio**

The effects of hen age and force molting programs on H: L ratio are illustrated in Table 4. No significant differences for H: L ratio between 63 week (0.50) and 75 week (0.51) age hens at the beginning of the molt period. There were no significant differences for H: L ratio among FW (0.50), A (0.49), AO (0.50), and O (0.52) treatments at the beginning of the molt period. At the end of the molt period, No significant differences in H: L ratio between 63 week (0.87) and 75 week (0.86) age hens were observed. However, hens molted with FW (1.08) have a significantly higher H: L ratio than hens

molted with A (0.74), AO (0.80), and O (0.83). No significant differences were observed in H: L ratio among nonfeed withdrawal treatments. This result agrees with the findings of Landers (2004) and Hnin et al (2009), who stated that hens molted by feed deprivation have a significantly higher H: L ratio when compared to birds that were not molted and birds molted by nonfeed withdrawal methods including alfalfa, rice, wheat or corn. However, Biggs et al (2004) stated that no differences were observed among feed removal treatments versus several nonfeed removal treatments including wheat and corn H: L ratios during the molt period.

**Table 4: Effects of Hen Age and Force Molting Programs on H: L Ratio during Molt Period**

|                  |    | H: L ratio      |                   |
|------------------|----|-----------------|-------------------|
|                  |    | Day 0           | Day 10            |
| Age (week)       | 63 | 0.50            | 0.87              |
|                  | 75 | 0.51            | 0.86              |
| SEM              |    | <b>0.01</b>     | <b>0.03</b>       |
| <i>P- value</i>  |    | <b>&gt;0.05</b> | <b>&gt;0.05</b>   |
| Molting Programs | FW | 0.50            | 1.08 <sup>a</sup> |
|                  | A  | 0.49            | 0.74 <sup>b</sup> |
|                  | AO | 0.50            | 0.80 <sup>b</sup> |
|                  | O  | 0.52            | 0.83 <sup>b</sup> |
| SEM              |    | <b>0.02</b>     | <b>0.05</b>       |
| <i>P- value</i>  |    | <b>&gt;0.01</b> | <b>&lt;0.01</b>   |
| 63 week          | FW | 0.50            | 1.06              |
|                  | A  | 0.46            | 0.73              |
|                  | AO | 0.48            | 0.83              |
|                  | O  | 0.54            | 0.84              |
| 75 week          | FW | 0.49            | 1.10              |
|                  | A  | 0.52            | 0.75              |
|                  | AO | 0.52            | 0.77              |
|                  | O  | 0.50            | 0.81              |
| SEM              |    | <b>0.02</b>     | <b>0.07</b>       |
| <i>P- value</i>  |    | <b>&gt;0.05</b> | <b>&gt;0.05</b>   |

FW: Feed withdrawal, A: Alfaalfa, AO: 50% Alfaalfa + 50% Oat, O: Oat  
SEM: Standard error of the mean

### Mortality

No death was observed in all treatment during the molt period. This result agree with by Aygun and Olgun (2010) and Aygun (2013), who reported that there were no death in FW and noon feed withdrawal treatments during molt period. The mortality was ranging from 1.20 to 8.3% among molt treatments during molt period in previous studies (Altan et al 1989; Biggs et al 2003; Petek and Alpay 2008; Aygun and Yetisir 2009).

### Conclusion

The results of this study indicated that hen age did not influence the body weight loss, egg production cease and stress as indicated by H: L during the molt period in our experimental conditions. The producer may be prefer to induced molting depend on egg prices between 63 and 75 week of age. In addition, nonfeed withdrawal methods can

be used successfully alternative to feed withdrawal methods for force molting because they have similar or smaller weight loss, similar egg production cease, and smaller H: L.

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