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Effects of Different Induced Molting Methods on the Performance of Commercial Layers

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Abstract: A total of 1600 commercial strain (Nick Chick) Single Comb White Leghorn hens, 90 week of age, were used in this study to determine the effects of different induced molting programs on production and immune parameters. The hens were randomly divided into four treatment groups (three experimental and one control) of 400 hens each. The hens in the first treatment group were fed a layer ration containing 4 g/kg diet of C for 5 d and received a reduced photoperiod of 8 h/d for 5 d (Al_2O_3 group). In the second group, feed was withdrawn for 10 d, the photoperiod was reduced to 8 h/d and oyster shell and water were provided for *ad libitum* consumption. At Day 11, hens consumed corn and oyster shell *ad libitum* until Day 30 and at Day 31, hens was returned to a full feed layer ration and received 16 h of light/d (California group). In the third treatment, birds were provided feed and water *ad libitum* for one day with 8 h light. During day 2-3, feed and water were withdrawn. On 4th day water was provided but no feed was given. On 5 to 49 days, birds were offered feed 27 g each till egg production was reached upto 1%. Water was provided *ad libitum*. Full feed at the rate of 112 g each bird was offered from day 50 onward. Birds received 8 h of light till 49 days which was increased to 16 h on 50th day onwards (Washington group). The last group served as control. Body weight, egg production, egg size, internal egg quality, shell weight and mortality were determined. Total circulating leukocytes and differential leukocyte counts were also measured. The results demonstrated that induced molting significantly increased egg production from 65 to 75 to 81%, Haugh units from 80.1 to 85.6 to 87.0 and shell weight from 5.4 g to 6.3 to 6.5 g when compared to control. The total circulating leukocytes was significantly lower in molted hens than in control hens. Differential leukocyte counts were affected by all induced molting programs and the heterophil to lymphocyte ratio was significantly increased, reaching 0.60, whereas that of controls was only 0.19.

Key words: California, Washington, aluminium oxide, performance, egg quality, immunity

INTRODUCTION

Molting is a major event in the annual life cycle of most avian species, both wild and domestic. Induced molting of the laying hens has been used extensively as a management tool for extending flock performance and in avoiding the annual cost of replacing pullets, vaccines, medicine and feed. Similarly, high mortality of the replacement pullets during the rearing period is also a major problem faced by the farmers as it affects the production and economics performance. Eggshell thickness, especially shell strength and quality decreases with age of hens (Rodriguez-Navarro *et al*., 2002) due to depletion of the calcium reserves in the body, yet post-molt shell thickness and eggshell quality was reported to have improved (Bar *et al*., 2001). Several procedures have been used to initiate molting. These include feed withdrawal up to 10 d (Christmas *et al*., 1985), water withdrawal for 2 d (North and Bell, 1990), photoperiodic reduction (Hembree *et al*., 1980), feeding low calcium (Breeding *et al*., 1992) or low sodium diets (Berry and Brake, 1985) and feeding dietary minerals (Khan *et al*., 2011). Induced molting by mineral supplementation, such as the use of high levels of either aluminium salt (Hussein *et al*., 1989) or dietary zinc (Hussein *et al*., 1988) has been successfully practiced. Each method can be used alone or in combination with other methods. All molting programs necessitate body weight loss and cessation of egg production (Yousaf and Chaudhry, 2008). In general, most researchers report that induced molting improves the postmolt performance of the laying hens compared to the premolt performance. The most important improvement is the increase in the rate of egg production during the postmolting period (Christmas *et al*., 1985).

Several studies have been conducted to investigate effects of individual molting programs on either production parameters or immune responses, direct comparisons of effects of the available molting programs, simultaneously, on both production parameters and immune responses are limited. Therefore, this study was designed to investigate,

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simultaneously, the effects of different induced molting programs on production, egg quality and immune parameters in laying hens.

MATERIALS AND METHODS

Experimental birds: A total of 1600 commercial strain (Nick-Chick) Single Comb White Leghorn hens, 90 week of age were used in the present study. The hens were randomly divided into four groups (three experimental and one control), housed on litter floor system at Breeding and Incubation Section, Poultry Research Institute, Rawalpindi. The hens received 16 h of light and 8 h of darkness/d. Feed and water were provided for *ad libitum* consumption prior to the beginning of the experiment. The composition of basal diet is given in Table 1. The experiment was conducted during the winter months.

Molting programs: The three molting programs used to compare their effects on production and immune parameters are described in Table 2. The programs included dietary aluminium oxide method (Al_2O_3), California method and Washington method. In the Al_2O_3 method described previously by Yousaf and Ahmad (2006), hens were fed a layer diet containing 4g/kg diet for Al_2O_3 for 5 d and received a reduced photoperiod of 8 h/d for 5 d. At Day 6, hens were returned to the control layer diet and received 16 h of light/d. The California method reported by Hurwitz *et al*. (1975) consisted of feed withdrawal for 10 d. During the withdrawal period, oyster shell and water were provided for *ad libitum* intake and the light period was reduced to 8 h/d. On Day 11, hens consumed cracked corn and oyster shell *ad libitum* until Day 30 and the light remained at 8 h/d. On Day 31, hens were returned to the control layer ration and received 16 h of light/d. In Washington method as described by North (1978), birds were provided feed and water *ad libitum* for one day with 8 h light. During day 2-3, feed and water were withdrawn. On 4th day water was provided but no feed was given. On 5 to 49 days, birds were offered feed 27 g each till egg production was reached upto 1%. Water was provided *ad libitum*. Full feed at the rate of 112 g each bird was offered from day 50 onward. Birds received 8 h of light till 49 days which was increased to 16 h on 50th day onwards. In order to determine the approximate minimum body weights that birds reach following molting, body weights were determined at the end of the treatment period (Al_2O_3) or at the end of the withdrawal period (California and Washington groups). Twenty five birds from each group were weighed at the beginning of the experiment, at Day 15 for the Al_2O_3 (end of the Al_2O_3 treatment) and the control groups, at Day 10 for all groups and at the peak of egg production for each group.

Parameters measured: Egg production and mortality were recorded daily starting at the beginning of the experiments and continued until 2 week after the peak of postmolt production for each treatment, including the period in which the birds were out of production. These periods were used to determine the days of stopping and returning to egg production. During the week of postmolt production of each treatment, 72 eggs were collected (36 eggs from the treated group and 36 eggs from the control group), weighed, shells were dried and weighed and the internal quality of the eggs was measured using Haugh unit values. Haugh units were calculated from the records of albumen height (measured with a caliper and recorded to the nearest 0.1 mm.) and egg weight (measured with electronic balance and recorded to the nearest 0.01 g) using the equation:

$$H.U. = 100 \times \log (\text{albumen height} + 7.57 - 1.7 \times \text{egg weight}^{0.37})$$

(Nesheim *et al*. 1979).

Total circulating leukocytes and differential counts were measured at the beginning of the experiment and at 5, 10 and 30 d following the start of the different molting programs and finally, at peak postmolt production. Cresyl blue and Hema 3 stains (Fisher Scientific, Pittsburgh, PA 15219) were used for determining total and differential cell counts, respectively. For differential counts, a total of 100 cells were counted and the number of each cell type was recorded.

Table 1: Composition of layer diet

Ingredients	g/kg
Corn	567.18
Soyabean meal	316.33
Vegetable Oil	76.82
Di-calcium phosphate	16.86
Calcium Carbonate	15.62
Methionine (98 %)	1.69
Vitamin premix[1]	2.50
NaCl	2.50
Trace mineral premix[2]	0.50
Total	1,000.00
Calculate analysis	
Dry matter	901.0
Crude protein	167.5
Ether extract	29.3
Crude Fiber	47.8
Ash	90.0
Calcium	30.0
Phosphorus (Available)	02.5
Metabolizable Energy (Kcal/kg)	2872.00

[1]Provided milligrams per kilogram of diet: vitamin A, 8.818 IU; vitamin D, 2.208 IU; vitamin E, 5.86 IU; vitamin K, 2.2 IU; thiamine, 1.1 IU; riboflavin, 4.4IU; niacin, 22 IU; choline, 500 IU; B12, 0.013 IU; Biotin, 0.055 IU

[2]Trace mineral premix provided milligrams per kilogram of diet: Mn, 68.2; Zn, 55; Cu, 4.4; I, 1.1; Se, 0.1

Statistical analysis: Data were analyzed using a one-way ANOVA to analyze the differences between treatment groups (Al_2O_3, California, Washigton and control) using the SPSS version 16 (SPSS, Cary, NC, USA) statistical analysis program. Means were compared using Duncan's Multiple Range Test (Steel and Torrie, 1984). Level of significance used in all results was ($p<0.05$).

RESULTS

Post molting layer performance: The effect of Al_2O_3, California, Washington and controls treatments on production parameters are shown in Table 3. The hens subjected to California method lost 22% of their initial body weight by Day 10 of the experiment, which was significantly greater than the loss in hens in the Al_2O_3 (16%) and Washington (15.6%) groups. Both Al_2O_3 and Washington groups lost significantly more weight than control hens. Hens subjected to Al_2O_3 treatment ceased egg production by Day 7 of the experiment and remained out of production until Day 12; Day 13 was the first day of egg production (Table 3). On the other hand, California hens ceased egg production on Day 8 and remained out of production until Day 42 of the experiment (Table 3). Hens subjected to the Washington method ceased egg production by Day 9 of the experiment and remained out of production until Day 53; Day 54 was the first day of egg production (Table 3). Hens reached 50% hen-day postmolt egg production by Days 18, 56, 66 for Al_2O_3, California and Washington groups, respectively, which was 2 to 4 week following the return to full-feed layer ration and 16 h of light/d. Furthermore, hens in all treatment groups reached peak production approximately 6 to 8 week following the return to full feed layer ration and 16 h of light/d. Hen-day egg productions during the peak week were 75, 81, 81 and 65% for Al_2O_3, California, Washington and control groups, respectively. The rate of egg production during the peak week of postmolt production for the molted groups was significantly higher than the control group (Table 3). In addition, the Al_2O_3 group had significantly greater egg production than control group but lower than the California and Washington treatments. The feeds efficiency during the second production cycle for the molted groups was significantly better than the control group (Table 3). The percentage mortality throughout the entire experiment for Al_2O_3, California, Washington and control were 3.5, 3.0, 2.5 and 4.5%, respectively (Table 3). The effect of Al_2O_3, California and Washington treatments on egg weight at the peak of postmolt production is shown in Table 3. No significant differences were found in egg weight between treatments. Eggs of hens subjected to Al_2O_3, California and Washington treatment had significantly higher shell weight during the week of peak production than eggs of 2 hens in the control group. Internal egg quality (Haugh units) was significantly higher in California and Washington methods than in the Al_2O_3 or control groups (Table 3).

Immunity: The effect of different induced molting programs on the total numbers of circulating leukocytes at different times of the experiment is shown in Table 4. There were no significant differences among treatments at the beginning of the experiment. On the 5th d of the experiment, total numbers of circulating leukocytes were significantly higher in all molted birds than in control birds (Table 4). On the 10th and 30th d of the experiment, California and Washington groups had a significantly higher number of circulating leukocytes than Al_2O_3 and control groups (Table 4). The total number of circulating leukocytes at the peak of postmolt production in the California and Washington groups was significantly higher than in the Al_2O_3 and control groups. The Al_2O_3 group had significantly higher total leukocytes than the control group (Table 4).

The effect of Al_2O_3, California, Washington and control on the heterophil:lymphocyte ratios at different time periods of the experiment is shown in Table 4. There were no significant differences among the treatments at the beginning of the experiment. On the 5th d of the experiment, heterophil:lymphocyte ratios were significantly higher for all molted groups than for the control group (Table 4). On the 10th d of the experiment, the ratios for California and Washington groups were significantly different from those of the Al_2O_3 and control

Table 2: Description of dietary Al_2O_3, California and Washington induced molting programs

Molting Program	Day of Experiment	Feed	Water	Light (h/d)
Al_2O_3	1 to 5	Full-feed layer diet containing 4 g Al_2O_3/kg	Provided	8
	6	Full-feed layer diet *ad libitum*	Provided	16
California[1]	1 to 10	None	Provided	8
	11 to 30	Corn *ad libitum*	Provided	8
	31	Full-feed layer diet *ad libitum*	Provided	16
Washington	1	Full-feed layer diet *ad libitum*	Provided	8
	2 to 3	None	None	8
	4	None	Provided	8
	5 to 49	27g feed layer diet till egg production was reached upto 1%	Provided	8
	50	Full-feed layer diet *ad libitum*	Provided	16

[1]Oyster shell is provided until Day 30

Table 3: Effect of different induced molting programs on production parameters of 90-wk-old White Leghorn laying hens

	Molting program			
Parameters	Control	Al_2O_3	California	Washington
Maximum % BW loss	+5	-16.0	-22.0	-15.6
Minimum BW reached, g	1,738[a]	1,507.3[b]	1,313.5[c]	1,600[b]
Last day of egg production	None	7	8	9
First day return to egg production from start of treatments	None	13	43	54
Day out of production	None	8	37	47
Day from beginning of treatment until returning to 50 to 60% production	None	18	56	66
Hen-day egg production during the week of peak production, postmolting, (%)	65[c]	75[b]	81.00[a]	81.00[a]
Feed Efficiency of molted layer in 2nd production cycle (feed /dozen eggs)	5.38[a]±0.153	3.08[b]±0.053	3.15[b]±0.044	3.28[b]±0.025
Mortality throughout the experiment, (%)	4.5	3.5	3.00	2.5
Egg weight at peak production, (g)	65±1.4	67.1±1.2	66.9±0.96	67.3±1.1
Shell weight at peak production, (g)	5.4±0.10[b]	6.2±0.12[a]	6.3±0.10[a]	6.5±0.18[a]
Haugh units at peak production	80.1±0.88[b]	81.0±1.7[b]	85.6±1.1[a]	87.0±1.3[a]

[a-c]Means with the different superscript within rows are significantly different ($p<0.05$)

Table 4: Effect of different induced molting programs on the total number of circulating leukocytes ($X10^3/mm^3$) and heterophil:lymphocyte ratios at different time periods of the experiment in 90-wk-old White Leghorn laying hens

	Molting program			
Parameters	Control	Al_2O_3	California	Washington
Total number of circulating leukocytes ($X10^3/mm^3$)				
At the beginning of experiment	58±2.4	58±2.4	58±2.4	58±2.4
On 5th d of experiment	58±1.9[b]	64±3.0[a]	74±4.0[a]	77±7.3[a]
On 10th d of experiment	56±3.0[b]	58±6.0[b]	69.0±5.9[a]	74±6.5[a]
On 30th d of experiment	58±2.3[b]	59±2.5[b]	79±7.0[a]	88±5.0[a]
At peak of postmolt production	57±2.4[c]	75±8.0[b]	90±5.0[a]	87±6.5[a]
heterophil:lymphocyte ratios				
At the beginning of experiment	0.16±0.02	0.16±0.02	0.16±0.02	0.16±0.02
On 5th d of experiment	0.15±0.03[b]	0.41±0.04[a]	0.35±0.38[a]	0.38±0.01[a]
On 10th d of experiment	0.19±0.02[c]	0.37±0.04[b]	0.60±0.05[a]	0.40±0.03[a]
On 30th d of experiment	0.16±0.02[c]	0.26±0.03[b]	0.38±0.03[a]	0.32±0.02[a]
At peak of postmolt production	0.17±0.03[b]	0.36±0.04[a]	0.40±0.01[a]	0.38±0.03[a]

[a-c]Means with the different superscript within rows are significantly different ($p<0.05$)

groups but not from each other. The California group had the highest heterophil:lymphocyte ratio followed by the Washington, Al_2O_3 and control groups (Table 4). On the 30th d of the experiment, ratios for California and Washington were significantly higher than for Al_2O_3 and control groups, whereas Al_2O_3 had significantly higher ratio than control group (Table 4). Heterophil:lymphocyte ratios at the peak of postmolt production in all treated groups were significantly higher than in the control group (Table 4).

DISCUSSION

Molt induction to rejuvenate the egg laying performance of commercial laying hen flocks is an important practice in many parts of the world, often being necessary to make a flock profitable under certain market scenarios. The first objective of an induced molt program is to cause hens to cease egg production and enter a non-reproductive state that cause body weight losses.

Post molting layer performance: In the present experiment, the rate of egg production was significantly improved by molting treatments when compared to control group. This result could be due to body weight loss as reported by Berry and Brake (1985) and Brake (1992), who indicated that the higher body weight loss, the higher postmolt production. Those authors also reported that induced molting leads to the involution of reproductive tract, which is proportional to the loss of body weight and that the rebuilding of the reproductive tract would lead to the removal of fat accumulation and therefore increased tissue efficiency. Another possible reason for improved egg production is the length of egg production cessation period. In the present study, although hens in California group lost more body weight than hens of the Washington group, both groups laid eggs at the same rate (81%) during the peak of postmolt production. This result could be due to the fact that hens in Washington group stayed out of production for a longer period of time (47d) than hens in California group

(37d). Furthermore, hens in the Al_2O_3 and Washington groups lost body weight to the same degree, although hens in the Al_2O_3 group laid at a lower rate (75%) than Washington hens (81%). This result could be because the Washington hens stayed out of production (47d) longer than hens in the Al_2O_3 group (8 d) and that resulted in greater egg production, as previously reported by Buhr and Cunningham (1994) and Bar *et al.* (2003). Those authors suggested that the longer the cessation period, the better the postmolt production. Within the different molting procedures, present results, in general, indicate that molting hens by the California or Washington methods allow a return to production at a higher rate than did the Al_2O_3 method.

In the present study, egg weight at the peak of postmolting production was numerically higher among the molted hens than in the control group, but the differences were not significant. This finding is in agreement with those of Christmas *et al.* (1985), Yousaf (2006) and Mejia *et al.* (2011) who found that different induced molting programs did not significantly affect egg weight when compared to the nonmolted birds. Although the egg weights were not affected, molting, using the Al_2O_3, California and Washington methods, increased shell weight at the peak of production. Improved shell gland function following induced molting may be due to remodeling at the cellular level. Cellular proliferation in the oviduct replaces cells lost during the regression, as evidenced by increased staining of the proliferating cells for proliferating cells nuclear antigen, a marker of cell proliferation (Heryanto *et al.*, 1997). Remodeling of shell gland tissue may also be responsible for removing substances that interfere with shell gland function. The uterine glandular epithelium which is the site of egg shell calcium transport and deposition contain quantities of intracellular lipid visibly detectable by histological staining (Baker *et al.*, 1981). Roland *et al.* (1977) reported that hens laying shell-less eggs have significantly higher uterine lipid levels compared to hens producing normal egg shells. Further reports by Baker *et al.* (1981) indicated that induced molting halts the incidence of shell-less eggs and removes lipid accumulation in the uterus. Berry and Brake (1987) reported that molting increases tissue receptivity to 1,25 (OH) dihydroxyvitamin D_3. The location of the cytosolic receptors for 1, 25 (OH) dihydroxyvitamin D_3 in shell gland glandular cells coincided with the reported location of calcium binding protein. Following the molt, intestinal calcium binding protein concentration increases compared to unmolted hens (Berry and Brake, 1987). Intestinal uptake of calcium also improves following molt (Al-Batshan *et al.*, 1994). Higher eggshells thickness of molted birds is a desirable characteristic for the egg industry (Keshavarz and Quimby, 2002). The improvement in shell quality could also be associated with the complete cessation of egg production during the molting period, as reported by Khan *et al.* (2011). The Haugh unit is a measure of the internal quality of an egg. Haugh units were higher for the Washington and California groups when compared to the control group. On the other hand, there was no improvement in egg quality from the hens in the Al_2O_3 group. These results are explained by the fact that Al_2O_3 group was out of production for the shortest period of time (8 d) compared to other groups, which did not allow for uterus or egg quality restoration to the degree realized for the California or Washington groups.

Immunity: In the present study, the leukocytes and heterophil:lymphocyte ratios, which are used to measure the level of stressful conditions (Manhiani *et al.*, 2011), were found to be higher in the molted groups than the control group. These results are in agreement with findings of Soe *et al.* (2007), who reported that on d 10 of molting, the H:L ratio of the control group was significantly ($p<0.01$) lower than that of the molted group. These results would indicate that molted birds were under more stress than control birds. Furthermore, it was found that total circulating leukocytes in the early stages of the molting period was reduced relative to those of the control hens. These results would imply that induced molting could result in inhibiting the immune response. Furthermore, throughout the present study, mortality for all molting groups was within the expected range, which suggests that excessive stress did not occur to influence either the immune response or livability.

Conclusion: The results indicate that induced molting is an effective way of bringing a dramatic recovery in both quality and rate of egg production without compromising the immunity of the bird. In addition, it could be concluded that the California and Washington programs were the best method for recovering both the rates of egg production and egg quality and the simplest procedure to be followed.

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