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Effect of Induced-Moult on the Number Small Ovarian Follicles and Egg Production of Old Layers

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Abstract: Influence of induced-moult on small ovarian follicles and egg production of old laying flock was investigated. Small follicles were graded thus: small yellow follicles (SYF), large white follicles (LWF) and small white follicles (SWF). A total of 360 old layers in their 64 weeks in lay were used in a 2x3 factorial arrangement in a Completely Randomized Design (CRD). The induced-moult treatments were natural day length with feed and water *ad libitum*, natural day length with water but no feed, natural day length with no feed and no water, reduced day length with feed and water *ad libitum*, reduced day length with water but no feed, reduced day length with no feed and no water, represented as T1, T2, T3, T4, T5 and T6, respectively. Each treatment was replicated 3 times with 20 hens per replicate. At the commencement of the experiment the numbers of the small follicles of the Control (T1) were 7.67 ± 0.88, 18.33 ± 0.88, 2121.67 ± 5.78, for small yellow, large white and small white follicles, respectively. The results showed that with the exception of T4, the numbers of all the small follicles of the rest of the induced-moult groups were significantly decreased ($P<0.05$) by day 7 of moult induction. The numbers of the small follicles of T2, T3, T5 and T6 gradually increased and became Significantly higher ($P<0.05$) than the control (T1) by day 49 of moult induction. By day 49, the numbers of the small follicles of the induced-moult hens ranged from 2500± 17.56 to 3670.00± 4.05 (SWF), 24.33±0.88 to 41.00± 0.58 (LWF) and 5.00± 0.58 to 6.67± 0.20 (SYF). The mean egg production of the flock was about 50 % prior to moult induction. The hen-day percent production of the moult groups ranged from 50 to 79 % whereas that of the unmoulted control ranged from 35 to 55 %. In conclusion, moulting initiated regeneration and rejuvenation of follicles. This in turn led to increase in post moult egg production of the induced-moult groups.

Key words: Egg production, ovarian follicles, induced-moult, layers

Introduction

The left ovary of a laying hen is a complex organ consisting of a cluster of follicles arranged in a hierarchy. Ovarian follicles are classified according to their size and diameter as large yolky follicles (LYF) (> 10mm), small yellow follicles (SYF) (5 to 9 mm), large white follicles (LWF) (2 to 4mm) and small white follicles (SWF) (< 2mm) (Etches, 1993). These small follicles supply the growing follicles for maintenance of the hierarchy. With the exception of the large yellow follicles, the rest of the follicles are regarded as small follicles. Fewer small follicles were found in the ovaries of aging hens and follicular atresia was common among these small follicles (Waddington *et al.,* 1985; Palmer and Bahr, 1992).

Follicles enter into rapid growth phase when they are less than 9 mm in diameter and contain white yolk (Gilbert, 1971). This confirmed the findings of Smith (1959) who reported that transformation of follicles from the resting stage to a rapid growth phase occurred around the diameter of 5 to 6 mm. The rate of follicular maturation was slower in aging birds than in young ones (Tanabe *et al.,* 1981; Moudgal and Razdan, 1984; Johnson *et al.,* 1986; Joyner *et al.,* 1987 and Palmer and Bahr, 1992). It is possible that changes that take place in the theca and granulosa tissues of the follicles transform the follicles from non-ovulable to ovulable stage in the hierarchy and that this could be responsible for follicular maturation. The mechanisms that are set in motion that cause the delay of the transformation of these follicles into rapid growth phase as hens advance in age are not fully understood. However, it was suggested that as laying hens age, longer time is taken in transporting yellow yolk into the small growing ovarian follicles (Gilbert, 1971; Williams and Sharp, 1978).

Etches (1996) reported that within 14 days of moult-induction, the population of large yellow follicles became reabsorbed and the ovary regressed, containing many small follicles. The differences in the sizes of yolky follicles, the changes in the way in which yolk accumulate into the follicles, the high rate of atresia in the small follicles and the slow recruitment of follicles into rapid growth phase between the old and young hens could be among the factors that initiate the gradual decline in egg production in a flock of aging birds. Su *et al.* (1995) reported increased number of small follicles following induced- moult of albino and non-albino old layers as well as increase in the growth intensity of the

follicles after induced-moult. Induced-moult was found to subsequently increase egg production from 64 % to between 77 % and 83 % (Alodan and Mashaly, 1999) when compared with the unmoulted control hens.

The objective of the study was to investigate the changes in the number of small ovarian follicles and subsequent egg production of old laying hens as influenced by induced moulting.

Materials and Methods

Location of study: The study was conducted in the Teaching and Research Farm of the College of Animal Science and Animal Health, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The University farm is geographically located on latitude 05 ° 29 ` North, Longitude 07°33 ` East and on Latitude of 122 meters above sea level. Umudike is within the rain forest zones of West Africa characterized by long periods of rainfall and short periods of dry season. The mean rainfall is 2169.8 mm in 148 to 155 days. The mean temperature is 26°C with minimum and maximum of 22 and 32°C, respectively. The relative humidity ranges from 50 to 95%.

Experimental design: The study was a 2x3 factorial arrangement in a completely randomized design with reduction in day length and feed/water withdrawal as induced moulting factors. Six experimental treatments in a completely randomized design were used in the study. These are: natural day length with feed and water *ad libitum*, natural day length with water but no feed, natural day length with no feed and no water, reduced day length with feed and water *ad libitum*, reduced day length with water but no feed, reduced day length with no feed and no water, designated as T1, T2, T3, T4, T5 and T6, respectively. The T1 served as the control. Each treatment was replicated three times with twenty birds per replicate.

Management of experimental animals: Three hundred and sixty 85-week old Isa Brown commercial layers were used for the study. The hens were managed in deep litter in a half-walled, well ventilated poultry house. The open sides were covered with wire mesh. Layer's diet, containing approximately 17% crude protein, and water were provided *ad libitum*, for 14 days prior to moult induction. The moult induction period lasted for 10 days during which period light, feed and water were restricted (for light restriction, day length was reduced to 8 hours per day). Following these 10 days, 50 days of recovery/ rest period were allowed to the induced-moult groups, which were maintained on moult ration of about 9% crude protein. Thereafter, all the experimental birds were returned to the initial layer's diet of 17 % crude protein and water *ad libitum* throughout the rest of the experimental period.

Measurements on ovarian follicles: Prior to moult induction (day 0), six birds sampled from the experimental population were slaughtered, their ovaries were removed and the different grades of follicles counted. This group of bird served as control (T1). Following slaughter, the follicles were incised, classified into sizes and the number of different classes of follicles counted. On days 7, 21, 35 and 49 following moult induction, six hens from each of the induced-moult groups were also sacrificed: their ovarian follicles were removed and counted.

Egg production records: Percent hen-day egg production was recorded prior to moult induction, during moult induction and over a period of 6 months post-moult.

Statistical analysis: Data generated were analyzed using analysis of variance (ANOVA). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

Results on the number of ovarian follicles of the experimental birds are presented in Tables 1, 2 and 3. There was a decrease in the number of the various classes of the follicles in the hierarchy following light, feed and water restrictions. With the exception of the group that was kept under reduced day length with feed and water *ad libitum* during the intensive period of moult-induction there was no record of large yellow follicles in the induced-moult groups The numbers of small follicles for the control (T1) group were 7.67 ± 0.88, 18.33 ± 0.88, 2121.67 ± 5.78, for small yellow, large white and small white follicles, respectively. Small yellow follicles of the induced-moult hens; natural day length with water but no feed, natural day length with no feed and no water, reduced day length with water but no feed, reduced day length with no feed and no water, (T2, T3, T5 and T6) were significantly lower ($P < 0.05$) than reduced day length with feed and water *ad libitum* (T4) on days 7 of moult–induction (Table 1). Also on days 35 and 49, small yellow follicles of all the forced moult groups showed no significant differences ($P>0.05$). However, small yellow follicles of T2, T3, T5 and T6 natural day length with water but no feed, natural day length with no feed and no water, reduced day length with water but no feed, reduced day length with no feed and no water), respectively showed progressive increase from day 21 through 49. The number of large white follicles of T2 (8.33), T3 (7.00), T5 (8.33) and T6 (8.67) (Table 2) were significantly lower ($P< 0.05$) than that of the control T1 (18.33) and T4 (18.00) on day 7. However, the reverse was the case by day 49. The number of large white follicles of T2, T3, T5 and T6

Table 1: Number of small yellow follicles of the birds in the induced-moult groups during the different days of moult-induction

Days	Treatment groups*				
	T2	T3	T4	T5	T6
7	1.67±0.67[a]	1.67±0.88[a]	6.00±1.00[b]	1.33±0.88[a]	1.33±0.33[a]
21	2.33±0.33[a]	2.00±0.58[a]	4.33±1.20[b]	2.33±0.33[a]	2.67±0.67[a]
35	2.67±1.67	3.00±0.58	3.00±0.00	3.33±1.33	3.33±0.48
49	5.00± 0.58	6.00±0.58	6.00±0.57	6.67±1.20	5.67±0.33

a-c Means in a row with different superscripts are significantly different (P<0.05)

* T2 = Natural day length with water *ad libitum*

T3 = Natural day length without feed and water

T4 = Reduced day length (8 hours of light) with feed and water *ad libitum*

T5 = Reduced day length (8 hours of light) with water *ad libitum*

T6 = Reduced day length (8 hours of light) without feed and water

Table 2: Number of large white follicles of the birds in the induced-moult groups during the different days of moult-induction

Days	Treatment groups*				
	T2	T3	T4	T5	T6
97	8.33±0.88[a]	7.00±1.00[a]	18.00±1.53[b]	8.33±0.88[a]	8.67±0.33[a]
21	16.00±1.00	17.67±2.00	16.00±2.00	16.00±0.58	16.33±2.40
35	21.67±2.19	23.00±2.52	19.33±0.33	26.33±0.88	24.33±2.03
49	41.00±0.58[b]	36.00±1.53[b]	24.33±0.88[a]	39.67±1.86[b]	40.00±2.52[b]

Foot note: as par Table 1

showed progressive increase from day 21 through 49 in a pattern similar to the small yellow follicles. The large white follicles of the control and the moult group placed under reduced day length with feed and water supplied *ad libitum* (T4) were significantly lower (P<0.05) than those of the induced-moult groups by day 49. Among the induced moult groups, the group placed under reduced day length with feed and water supplied (T4) recorded significantly lower values in large white follicles on day 49. Progressive increase was observed in the number of small white follicles of the induced-moult groups after re-feeding during the period of moult - induction. This confirms the results of Su *et al*. (1995) and Etches (1996) who observed that within 14 days of moult induction, large yellow follicles were absorbed and the regressed ovary contains many small follicles. However, the observed increase in the white follicles in the induced – moult group placed under reduced day length with feed and water *ad lib* (T4) was less pronounced than the other induced - moult groups. In the control group, the number of white follicles gradually decreased contrary to the other groups. This gradual decline in the small and large white follicles of the control birds could be attributed to their not being moulted as they advanced in age or to high incidence of follicular atresia among these small follicles in aging birds (Waddington *et al.,* 1985; Joyner *et al.,* 1987 and Palmer and Bahr, 1992). The significantly (P<0.05) increased number of the large white follicles at about 49 days of moult induction in the induced moult groups suggested recruitment of follicles from the pool of regenerated small white follicles.

The gradual decline in the yellow follicles of the group under reduced day length with feed and water *ad libitum* (T4) is attributable to the procedures used in the moult - induction of this group (light restriction only and feeding of moult ration). This procedure may not have been stressful enough as to induce complete moult that is required for regression and rejuvenation of follicles. Alternatively, the decrease or the prolonged rejuvenation of the small follicles of the later group may be due to the effects of the low protein levels of the moult diet since this group laid more eggs than the control after the induced - moult periods. The significant decrease in the small yellow follicles of the different induced - moult groups by day 7 of moult-induction and increase by day 49 to approximately their original number could be attributed to the changes in the patterns of redevelopment of yellow follicles. The significant effects of feed and water withdrawal on the greater majority of the gradation of follicles confirms that feed and water withdrawal are the major moult-inducing methods in moulting programmes.

The effects of day length, feed and water withdrawal significantly reduced the number of small yellow follicles of the induced - moult groups but there was increase in numbers of the small white follicles. The large white follicles and small yellow follicles are recruited from the small white follicles during follicular growth and

Table 3: Number of small white follicles of the birds in the induced-moult groups during the different days of moult-induction

Days	Treatment groups*				
	T2	T3	T4	T5	T6
7	1110.00±4.58	1120.00±4.04	1326.69±3.28	1103.33±4.84	1086.67±0.33
21	2426.67±14.44	2463.33±15.06	2280.00±9.45	2683.33±14.24	2496.67±19.27
35	3020.00±27.15^{b}	2960.00±42.06^{b}	2173.33±9.33^{a}	3223.33±29.25^{b}	3153.33±12.45^{b}
49	3426.67±17.03^{b}	3456.67±13.86^{b}	2850.00±17.56^{a}	3473.33±31.92^{b}	3670.00±14.05^{b}

Foot note: as par Table 1

Table 4: Hen day percent egg production of the birds during the post-moult period

Months	Treatment groups*					
	T1	T2	T3	T4	T5	T6
1	48.80±1.72^{b}	31.16±2.76^{a}	29.13±1.51^{a}	31.62±1.24^{a}	30.55±1.28^{a}	26.16±1.45^{a}
2	47.37±1.96^{a}	68.07±2.39^{b}	74.34±3.59^{b}	67.41±3.53^{b}	74.94±1.89^{b}	68.40±1.38^{b}
3	43.94±1.59^{a}	72.33±2.05^{c}	71.60±2.67^{c}	63.85±1.83^{b}	74.79±2.14^{c}	71.59±2.31^{c}
4	44.33±1.59^{a}	70.75±0.40^{bc}	75.06±0.65^{cd}	65.40±2.29^{b}	79.32±1.56^{d}	70.45±1.26^{b}
5	41.78±3.15^{a}	67.98±3.74^{bc}	70.05±1.56^{c}	61.79±2.31^{b}	72.97±1.61^{c}	65.47±1.26^{bc}
6	42.71±3.26^{a}	67.34±3.84^{b}	61.33±2.68^{b}	50.20±3.02^{a}	69.79±2.62^{c}	69.52±2.19^{c}

maturation. This lends supports to the findings of Bai *et al*. (1994) who indicated that induced moult causes old layers to enter into another stage of their life. Thus a second laying cycle is initiated following follicular regeneration and rejuvenation achieved by induced moult.

Egg production: Egg production stopped in the induced-moult groups T2, T3, T5 and T6 between 3 to 6 days of moult induction. These hens resumed egg production between 21 and 30 days after cessation of lay following moult-induction. Resumption of lay suggests that the ovaries retained their state of responsiveness during moult induction regardless of the length of the moult periods. Results on the mean egg production of the experimental birds are presented in Table 4. The average hen-day percent post moult egg production of the moulted groups in months 2 to 6, ranging from approximately 50 to 79 % was significantly greater ($P<0.05$) than that of the unmoulted control (42 to 47 %). This confirms the frequently reported improvement in egg production during post - moult periods (Odunsi *et al*., 2002; Bell, 2003).

Although there were no significant differences in the length of time taken to resume egg laying in the induced-moult groups, the group under natural day length with water (T2), resumed earlier than the rest. The induced-moult groups have peak egg production ranging between 67 and 79 % whereas the control showed progressive decrease (42%) in egg production following the period of moult-induction.

The differentials observed in the attainment of peak production could be attributed to the rate of rejuvenation and recruitment of small white follicles into large white follicles during the rapid growth phase. It could also be due to the rate of yolk synthesis by the liver or other physiological and/or endocrinological mechanisms that were unnoticed during the study. The groups under natural day length without feed and water (T3), and reduced day length with water (T5) showed greater persistency in laying than the other induced-moult groups after peak production. The induced–moult group kept under reduced day length with feed and water *ad libitum* (T4) showed significant decrease to about 50.20 % after peak of egg production. On the other hand, the rest of induced-moult groups continued production at higher economic rates of about 61.33 to 69.79 % up to 6 month after moult-induction.

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