African Journal of Biotechnology Vol. 4 (9), pp. 1005-1009, September 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Plasma progesterone profile and ovarian activity of forced-moult layers

M.A. Oguike[1] G. Igboeli[2] S.N. Ibe[1] Iromkwe M.O.[1] Akomas S.C[1]. and M. Uzoukwu[1]

[1]Michael Okpara University of Agriculture, Umudike, P.M.B. 7267. Umuahia, Abia State, Nigeria.
[2]Department of Animal Science, University of Nigeria, Nsukka, Nigeria.

Accepted 26 July.2005

Different techniques of moult induction were used to force moult 360 commercial old layers, aged 85 weeks. The techniques 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, designated as T1, T2, T3, T4, T5 and T6, respectively. The T1 served as the control. Sixty hens were randomly assigned to each treatment which was replicated 3 times. The moult induction period was for 10 days coupled with 50 days of recovery period when the birds were fed low protein moult diet. At day 7, the ovaries of T2, T3, T5, T6 regressed weighing 3.43, 7.03, 5.00, 4.80 g, respectively. These were significantly (P<0.05) lower than the ovarian weights of 34.73 and 35.13 g of T4 and control (T1), respectively. By day 35 of moult induction, the ovaries of T2, T3, T5 showed the greatest recovery increasing to 18.53, 20.73, 13.27 g, respectively, while T4 decreased to 13.00 g. The number of large yellow follicles of T2, T3, T5, T6 decreased from 3.33 on day 0 to 0.00 on day 7. By day 21 the large yellow follicles of T2, T3, T5 and T6 started regenerating, ranging between 2.33 and 3.00 and by day 49 were significantly (P<0.05) higher than T4 (1.67). Plasma progesterone levels decreased from between 0.50 and 0.60 ng/ml on day 0 to undetectable levels by days 7 and 14 in T2, T3, T5, T6. By day 21, plasma progesterone levels (ng/ml) started rising in T2 (0.40), T3 (0.33), T5 (0.40), T6 (0.33) although these were significantly (P<0.05) lower than those of T1 (0.77) and T4 (0.81). As the number of large yellow follicles increased, the concentration of progesterone in the plasma increased.

Key words: Layers, forced-moult, ovarian follicle, progesterone.

INTRODUCTION

Ovarian function of the hen depends on the information relayed from the hypothalamo-hypophyseal axis as well as from the ovarian tissues themselves. In laying hens, the primary sources of progesterone are the granulosa cells of the five largest follicles in the hierarchy (Verheyen et al., 1987). These ovarian follicles contribute the major part of pre-ovulatory peak of progesterone (Etches, 1984). Therefore, progesterone is the only steroid produced by the ovulating follicle (Imai and Nalbandov, 1978; Decuypere et al., 1993).

Natural moult occurs with advancement in the age and length of intensive production of a laying flock. The effects of age on plasma concentration of progesterone were studied by Joyner et al. (1987) who recorded progesterone levels of 4.29, 3.80 and 1.64 ng/ml for pullets, old layers and old non-laying hens, respectively. However, Etches (1996) indicated that moulting occurs when plasma concentrations of steroids and gonadotropins are low. This is in agreement with the findings of Decuypere and Verheyen (1986) and Jacquet et al. (1993) who reported that changes in the

*Corresponding author. E-mail: maoguike2000@yahoo.com.

reproductive functions during forced-moulting were associated with reduced levels of luteinizing hormone and sex steroids. The major changes caused by moulting within the hypothalamo-hypophyseal-gonadal axes result in temporary cessation of lay.

Moult induction by reduction in day length and starvation precipitate significant decrease in sex steroids and gonadotropins. Etches (1996) reported that within 14 days of moult induction, the large follicles were reabsorbed and the regressed ovary was left with many small follicles. This confirmed the works of Su et al. (1995) who reported increased number of large white follicles in the ovaries of albino and non-albino hens following forced-moult. Follicular atresia was reported to be partly due to refractoriness of the hypothalamus and probably also the hypophysis to progesterone stimulation during fasting (Johnson and Van Tienhoven, 1980). Tanabe et al. (1981) reported decreased plasma levels of progesterone and oestradiol in laying hens during starvation. They also observed that plasma progesterone levels ranged between 0.51 and 0.72 ng/ml during starvation, in contrast with 1.38 ng/ml when fed *ad libitum*. Guemene and Williams (1992) recorded variations of plasma progesterone levels of 0.93, 0.25 and 0.32 ng/ml in laying, incubating and out-of-lay hens, respectively.

The objective of the study, therefore, was to investigate changes in plasma progesterone levels and the physiological processes associated with ovarian activities of forced-moult layers.

MATERIALS AND METHODS

Experimental animals and treatments

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. 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 conventional half-walled, properly ventilated poultry house typical of a tropical environment. The open sides were covered with poultry wire mesh. The hens were randomly allotted to six experimental treatments in a completely randomized design, as follows: 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 giving a total of 60 birds per treatment. Layer's diet, containing about 17% crude protein, and water were provided *ad libitum,* except during the moult induction period. The moult induction period consisted of 10 days of light, feed and water restrictions and 50 days of recovery/rest period. Day length was reduced to 8 h per day using black polythene materials. During the 50 days of recovery, the forced-moult groups were maintained on moult ration of about 9% crude protein.

Parameters measured

Prior to moult induction, six birds sampled from the experimental population were slaughtered and their ovaries were removed and weighed. The ovarian weights were taken using Mettler weighing balance as soon as they were incised. Also following slaughter, the large yellow follicles were incised and the number of follicles counted. On days 7, 21, 35 and 49 following moult induction, six hens from each of the forced-moult groups were also sacrificed, their ovaries removed and weighed while the large yellow follicles were removed and counted. Blood samples (2.5 ml) were obtained between 0900 and 1100 h from six birds from each treatment group on days 0, 7, 14, 21, 28, 35, 42, 49 and 56. Samples were obtained from the wing vein into heparinised tubes. Plasma was separated from blood cells by centrifugation at 350 cycles g^{-1} for 30 min and kept below 0°C in a freezer until required for assay of progesterone. Blood samples collected from birds of the same treatment group on each sampling day were pooled. Progesterone concentrations in the plasma of experimental birds were determined using Radioimmunology assay (RIA) kits. The kits were Progesterone RIA pre-coated tubes prepared by the Animal Production Unit, Agricultural Laboratory, Agency's Laboratories, Seibersdorf, Austria, in collaboration with Diagnostic Products Cooperation, Los Angeles, USA, in support of the Joint FAO/IAEA Programme in Animal Production and Health.

Statistical Analysis

Data generated were analyzed using analysis of variance (ANOVA). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955). All statistical analyses were done.

RESULTS

Ovarian weights of birds in the different treatment groups are presented in Table 1. The ovarian weight of the T4 group (reduced day length with feed and water *ad libitum)* was significantly ($P<0.05$) higher than those of the other groups 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) on days 7 and 21. The mean ovarian weight of the control birds T1 (natural day length with feed and water *ad libitum*) sampled on day 0 was 35.13±0.94 g and was significantly higher ($P<0.05$) than all the different groups throughout the period of study. For the forced-moult groups, the ovarian weight showed a sharp decline on day 7 and this was most severe (about 3.43 g) in the T2 group followed by T6 (4.80 g). The ovaries of the forced-moult groups atrophied following moult induction. By day 7, the ovarian weights of the forced-moult groups 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) ranging from 3.43 to 7.03 g were significantly ($P<0.05$) lower than that of T4 (the group with reduced day length with feed and water *ad*

Table 1. Ovarian weight (g) of the birds in the treatment groups during the different days of moult-induction.

Days	Treatment groups				
	T2	T3	T4	T5	T6
7	3.43±0.12[a]	7.03±0.87[a]	34.73±1.08[b]	5.00±0.12[a]	4.80±0.06[a]
21	9.70±0.44[a]	4.83±0.17[a]	22.83±0.72[b]	3.63±0.17[a]	3.33±0.09[a]
35	18.53±0.84[bc]	20.73±0.83[c]	13.00±1.69[ab]	.27±0.13[ab]	11.80±1.35[a]
49	15.07± 0.80[ab]	18.10±0.60[b]	16.87±0.79[b]	10.17±0.58[a]	16.47±0.35[b]

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 h of light) with feed and water *ad libitum.*
T5 = Reduced day length (8 h of light) with water *ad libitum.*
T6 = Reduced day length (8 h of light) without feed and water.

Table 2. Distribution of the number of large yellow follicles of the birds in the treatment groups during the different days of moult-induction.

Days	Treatment groups				
	T2	T3	T4	T5	T6
7	0.00±0.00[a]	0.00±0.00[a]	3.33±3.33[b]	0.00±0.00[a]	0.00±0.00[a]
21	.00±0.58[a]	1.67±0.33[a]	2.67±0.88[b]	1.00±0.58[a]	1.00±0.08[a]
35	2.00±1.00	3.33±0.33	2.33±0.33	2.00±0.58	2.00±0.58
49	2.33±0.33[b]	3.00±0.58[b]	1.67±0.88[a]	2.33±0.33[b]	2.67±0.33 [b]

a, b: Means in a rows 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 h of light) with feed and water *ad libitum.*
T5 = Reduced day length (8 h of light) with water *ad libitum.*
T6 = Reduced day length (8 h of light) without feed and water.

libitum, 34.73 g). However, by this period, the ovarian weights of the forced-moult groups (placed under natural day length with water, natural day length without feed and water, reduced day length with water and reduced day length without feed and water [T2, T3, T5 and T6, respectively]), were not significantly (P>0.05) different from each other. The ovaries of T2, T3, T5 and T6 had the greatest recovery at day 35. The ovary of the T4 group showed continuous decline in weight from day 7 to day 35 but slightly increased by day 49.

The number of large yellow follicles is presented in Table 2. On day 7, the number of large yellow follicles in the control sample and T4 was 3.33 and there were no large yellow follicles in the ovaries of the forced-moult groups T2, T3, T5 and T6. The large yellow follicles of T2, T3, T5 and T6 started regenerating by day 21. From day 21 through day 49, the number of large yellow follicles of T4 group decreased progressively. By day 49, the number of follicles in T2 (2.33), T3 (3.00), T5 (2.33) and T6 (2.67) were significantly (P<0.05) higher than T4 (1.67).

The plasma concentration of progesterone in the different treatments is shown in Table 3. On days 7 and 14 of moult induction, the hens in T2, T3, T5, and T6 recorded undetectable levels of progesterone in their plasma, whereas the level in T1 (control) was significantly (P<0.05) highest followed by T4. The plasma levels of progesterone of T1 and T4 were also significantly higher (P<0.05) than T2, T3, T5 and T6 on day 21. Thereafter, the progesterone levels started to rise in all the forced-moult groups although plasma progesterone levels fluctuated in all the groups throughout the experimental period. There were no significant differences (P>0.05) in the progesterone levels of the different treatment groups on days 42, 49 and 56.

DISCUSSION

The results of this study indicate that changes in ovarian weight and large yellow follicles were associated with reduction of plasma concentrations of progesterone. This observation is consistent with the reports of Decuypere and Verheyen (1986) and Jacquet et al. (1993). This phenomenon could be explained by the absence of the large yellow yolk filled follicles in the ovaries of the forced-moult groups. This could also be due to the severity of the effects of feed and water removal as well

Table 3. Plasma progesterone levels (ng/ml) of the different group of birds during moult-induction period.

Days	Treatment groups*					
	T1	T2	T3	T4	T5	T6
0	0.60±0.12	0.57±0.17	0.50±0.06	0.60±0.06	0.57±0.12	0.60±0.15
7	0.63±0.12^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.33±0.03^{b}	0.00±0.00^{a}	0.00±0.00^{a}
14	0.74±0.09^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.33±0.03^{b}	0.00±0.00^{a}	0.00±0.00^{a}
21	0.77±0.09^{b}	0.40±0.06^{a}	0.33±0.03^{a}	0.81±0.16^{b}	0.40±0.06^{a}	0.33±0.03^{a}
28	0.63±0.15^{b}	0.63±0.05^{b}	0.40±0.06^{a}	0.70±0.17^{b}	0.50+0.01^{b}	0.37±0.07^{a}
35	0.71±0.22^{c}	0.50±0.30^{b}	0.40±0.06^{a}	0.53+0.07^{b}	0.40±0.11^{a}	0.50±0.03^{b}
42	0.90±0.36	0.61±0.16	0.67±0.26	0.77±0.42	0.69±0.19	0.83±.39
49	0.83±0.58	0.77±0.03	1.03±0.19	0.83±0.03	0.93±0.03	0.70±0.06
56	0.80±0.17	1.01±0.06	0.84±0.03	0.93±0.32	1.03±0.33	0.97±0.18

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 h of light) with feed and water *ad libitum.*
T5 = Reduced day length (8 h of light) with water *ad libitum.*
T6 = Reduced day length (8 h of light) without feed and water.

as reduction in day length on ovarian follicular hierarchy. The greatest recovery of the ovaries of the forced-moult groups T2, T3 and T5, by day 35 could be attributed to rejuvenation processes associated with forced-moult and/or compensatory growth following re-feeding.

The control and the moult group under reduced day length with feed and water *ad libitum* (T4) contained some large yellow follicles in their ovaries and had some progesterone in their plasma. There are several reports that in laying hens, the granulosa cells of the three largest yellow yolky follicles in the ovarian follicular hierarchy are the primary sources of progesterone (Decuypere and Verheyen, 1986; Joyner et al., 1986; Verheyen et al., 1987; Guemene and Williams, 1992; Jacquet et *al.,* 1993; Su et al., 1995; Etches, 1996 and Rose, 1997). This could also be an explanation for the increased progesterone levels in birds having greater number of large yellow follicles. Thus, the rising levels of plasma progesterone in the forced-moult groups by day 21 are in agreement with the redevelopment and/or regeneration of the large yellow follicles in the ovary at this time. Furthermore, as the numbers of the large yellow follicles increased, the concentrations of progesterone in the plasma increased. This observation is true of the T2 T3, T5 and T6 on day 21, thus confirming that changes in progesterone production are fundamentally indicative of the regeneration and/or growth of the follicles in the ovary. The differences in the plasma progesterone levels of the different forced-moult groups suggested and/or reflected differences in their follicular growth and maturation. Significantly reduced plasma levels of progesterone in the forced-moult groups, T2, T3, T5 and T6 (natural day length with water, natural day length without feed and water, reduced day length with water and reduced day length without feed and water, respectively) by day 7 of moult-induction also indicates that starvation affects plasma progesterone levels.

However, there could be other mechanisms responsible for these changes in plasma progesterone levels. Deprivation of feed and water could be responsible for significant decrease in plasma concentrations of progesterone to non-detectable 1evels, since the levels gradually increased following re-feeding. This confirms the results of Verheyen et al. (1987). The plasma progesterone concentrations of the birds in this study fall within the levels (0.25 to 0.93 ng/ml) reported by Guemene and Williams (1992) but below the levels (1.64, 3.80 and 4.29 ng/ml) reported by Joyner et al. (1986) for laying pullets, old layers and non-laying old layers, respectively. The differences between the levels recorded by Joyner *et al.* (1986) and present study could be attributed to environmental differences or other unknown factors.

In conclusion, moulting induced physiological stress resulted in regression of the ovarian tissues. This in turn led to reduction of ovarian activities with decrease in progesterone production. However, the regeneration of large yellow follicles secretion of progesterone commenced after moult induction period. These changes indicated the rejuvenation processes and the reactivation of ovarian tissues associated with forced-moult.

ACKNOWLEDGEMENTS

The authors are indebted to the Senate Research Grant Committee of Michael Okpara University of Agriculture Umudike, for funding this research. They are also grateful to Professor E.O Oyedipe, Dr. L.O. Eduvie and Mr. J.

Iyayi through whose assistance the assay of progesterone was done with the laboratory facilities of National Animal Production Research Institute (NAPRI), Zaria, Nigeria.

REFERENCES

Decuypere E, Verheyen G (1986). Physiological basis of induced-moulting and tissue regeneration in fowls. World's Poult. Sci. 42: 56 – 68.

Decuypere E, Leenstra F, Huybrechts LM, Feng PY, Arnonts S, Herremans M, Nys M (1993). Selection for weight gain or food conversion in broiler affects the progesterone production capacity of large follicles in reproductive adult breeders Brit. Poult. Sci. 34: 543–552.

Duncan DB (1955). Multiple Range Test. Biometrics 11: 1-42.

Etches RJ (1996). Reproduction in Poultry. CAB International, Wallingford, UK. pp. 267-397.

Etches RJ, Duke CE (1984). Progesterone androstenedione and oestradial content of theca and granulosa tissues of four largest ovarian follicles during ovulatory cycle of hen. J. Endocrinol. 103: 71-76.

Guemene D, Williams JB (1992). Comparison of the basal and luteinizing hormone-releasing hormone induced luteinizing hormone release by perifused hypophyses from turkey hens (*Meleagris gallopavo*) at different physiological stages. Brit. Poult. Sci. 33: 153-163.

Imai K, Nalbandov AV (1978). Plasma and follicular steroid levels of laying hens after the administration of gonadotropins. Biol. Reprod.19: 779-784.

Jacquet JM, Seigneurin F, De Rivers M (1993). Induced-moulting in cockrels Effect on sperm production, plasma concentration of luteinizing hormone, testesterone and thyroxine on pituitary sensitivity to luteinizing hormone-releasing hormone. Brit. Poult. Sci. 34: 765-775.

Johnson AL, Van Tienhoven A (1980). Hypothalamo-hypophyseal sensitivity to central injection of progesterone and luteinizing hormone-releasing hormone in the laying and moulting hen. Adv. Physiol. Sci. 33: 99-107.

Joyner CJ, Peddie MJ, Taylor TG (1987). The effect of age on egg production in the domestic hen. Gen. Comp. Endocrinol. 65: 331-336.

Rose SP (1997). Principles of Poultry Science. CAB. International. Wallingford.UK. pp. 44-77.

Su H, Silversides FG, Villeneuve P (1995). Ovarian morphology and follicular development in sex-linked imperfect albino (s^{al-s}) and non albino hens before or after a forced-moult. Br. Poult. Sci. 35: 645-653.

Tanabe Y, Ogowa T, Nakamura T (1981). The effect of short-term starvation on pituitary and plasma luteinizing hormone, plasma oestradiol and progesterone, and pituitary response to luteinizing hormone-releasing hormone in the laying hen (*Gallus domesticus*). Gen.Comp. Endocrinol. 43: 392-398.

Verheyen G, Decuypere E, Chiasson RB, Vervloesem J, Kuhn ER, Michels H (1987). Effect of exogenous luteinizing hormone on plasma concentration of progesterone and oestradiol in relation to the cessation of egg laying induced by different moulting methods. J. Reprod. Fertil. 81: 13-21.