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Effects of human menopausal gonadotrophin (HMG) on plasma hormonal profile and egg production in local guinea hens

Alli, O. I., Toye, A. A., Adeyina, A. O., Ayorinde, K. L., Okukpe, K. M., and Ajao, B. H.

Department of Animal Production, University of Ilorin, Ilorin, Nigeria.

Corresponding Author: ibidapoalli@gmail.com ***Phone No.:*** 08030607225

Target Audience: Farmers, researchers

Abstract

Commercial production of guinea hens is limited because they are known to be seasonal breeders, with little or no eggs during the dry season. Natural hormones or their analogue have gained special appeal and usage as means of improving the reproductive performances of farm animals due to the health concerns associated with use of synthetic hormones. Human menopausal gonadotrophin (HMG) consists of follicle stimulating hormone (FSH) and luteinizing hormone (LH) and is aimed at improving reproductive performance. Five doses (0, 6, 12, 18 and 24 IU) of HMG were administered to guinea hens in two phases (dry and rainy seasons) to evaluate the effect on the follicle stimulating and luteinizing hormone profile and egg production for a period of eleven months. The effects were monitored during the administration and post administration periods. Egg collection was done daily and blood was collected from the birds on fortnight basis to determine concentration of FSH and luteinizing hormone LH. Data collected were subjected to analysis of variance appropriate for 2x2x5 factorial design. LH and FSH concentrations were correlated with egg production. Results indicated that both LH and FSH concentrations were higher ($P<0.05$) in the rainy season and during the period of post administration of HMG while doses of HMG had no effect ($P>0.05$) on the concentration of both hormones. LH and FSH were positively correlated in both seasons but the correlation was highly significant ($P<0.01$) only in the rainy season. Low negative correlation existed between egg production and LH in the dry season while positive, low and non-significant ($P>0.05$) correlation was observed in the rainy season. In conclusion, administration of HMG could be used to boost egg production in guinea hens.

Key words: *guinea hen, follicle stimulating hormone, hen day production, luteinizing hormone*

Description of Problem

Recently, natural hormones or their analogue have found special appeal and usage as means of improving the reproductive performances of farm animals (1). This has become important because of the health concerns of using synthetic hormones. The principal target is to increase both Luteinizing hormone (LH) and follicle stimulating hormone (FSH) secreted by the anterior pituitary gland and which are both essential in reproduction in animals. While the follicle stimulating hormone is involved in the development of tiny follicles, the luteinizing hormone stimulates the production of sex steroids in the ovarian follicles (2,3).

Since the first experimental induction of super ovulation was reported (4), several commercial preparations of gonadotropins have been used in superovulatory protocols.

Induction of super-ovulation in beef heifers and ewes with Human Menopausal Gonadotropin (HMG) have been reported (5,6). Human Menopausal Gonadotropin, also called menotropin, consists of luteinizing hormone and follicle stimulating hormones in the ratio 1:1 (7) and is extracted from the urine of women who have reached menopause. It may also contain human chorionic gonadotropin, HCG (8). HMG is used to stimulate production of FSH and LH and is originally designed to make women more fertile by stimulating the ovaries to produce multiple eggs.

Wild and semi domesticated guinea fowls are seasonal egg producers and lay only few eggs each season. The hens have been reported to lay more eggs during the raining season with production declining towards the end of the season and zero production during the dry season (9, 10). This to a great extent has limited the commercial production of guinea fowls. Therefore, every effort to improve their egg laying capacity through the usage of safe, natural and non-abusive source of hormonal preparations would therefore be of advantage. The study was therefore carried out to investigate the influence of HMG on the hormonal profile and egg production of guinea hens.

Materials and Methods

Birds and Management

The study was carried out at the Animal Pavilion of the Department of Animal Production of the University of Ilorin, Ilorin. A total of 125, thirty-six weeks old local guinea hens selected from an existing base population were used for the experiment; each treatment (dose of HMG) was replicated five times, with five birds per replicate. The birds were randomly allocated to five treatment groups (doses) covering two seasons (dry and raining seasons) and two efficacy periods (period of HMG administration and post administration period) in a 2 x 2 x 5 factorial design. The HMG used in the study was purchased from a reputable pharmaceutical firm in Lagos, Nigeria. The treatment groups were:

1. 24.0 IU (0.32ml) of HMG per bird
2. 18.0 IU (0.24ml) of HMG per bird
3. 12.0 IU (0.16ml) of HMG per bird
4. 6.0 IU (0.08ml) of HMG per bird
5. 1.0 ml of physiological saline solution (control).

The HMG (IVF-M $_{Inj}$) was administered intramuscularly on three consecutive days per week (Monday to Wednesday) for six weeks during the dry season (December/January) following which the withdrawal effect was monitored over four months. The administration was repeated for six weeks during the rainy season (May/June) and the withdrawal effect also monitored over a period of four months. Birds were housed in battery cages and fed diet containing 20% crude protein and 2750kcal/kg metabolizable energy. Feed and water were supplied *ad libitum* throughout the experimental period which lasted 11 months.

Data collection

Egg Production: Total number of eggs (TEN) laid in a week per bird was recorded and the hen day production (HDP) which is total number of eggs laid by the flock in a given period divided by the product of the number of days and the number of hens alive on each of these days, was calculated using the formula:

$$\text{HDP (\%)} = \frac{\text{Number of eggs produced}}{\text{Number of hens alive during the period the eggs were collected}} \times 100$$

Hormonal Assay: Blood was collected fortnightly by drawing blood from the wing web of birds in each replicate and pooled into sample bottles containing EDTA to prevent the blood from clotting. Concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were determined on fortnight

basis using a commercial kit, Enzyme Linked Immunosorbent Assay (ELISA) by TECO Diagnostics®.

Changes in Reproductive Tract Size: From each treatment group, the reproductive tract of four randomly selected slaughtered hens was carefully removed. The ovaries were weighed on a sensitive weighing scale and the length of the oviduct measured with a measuring tape. The ova were harvested and graded according to their dimension into three categories viz the large yellow follicles (greater than 8mm in diameter), the small yellow follicles (between 2 and 8 mm in diameter) and the large white follicles (between 2 and 5 mm in diameter), using a light microscope.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) appropriate for a 2 x 2 x 5 factorial using Genstat Discovery Edition 4. Differences in means were separated using Duncan multiple range test of the same statistical package. LH and FSH concentrations were correlated with egg number using the formula:

$$r = \frac{\sum (xy)}{\sqrt{[(\sum x^2) * (\sum y^2)]}}$$

Where:

Σ is the summation symbol,

$x = x_i - \bar{x}$,

x_i is the x value for observation i,

$\bar{x}$ is the mean x value,

$y = y_i - \bar{y}$,

y_i = y value for observation i, and

$\bar{y}$ = mean value of y.

Results

Hormonal Profile of Guinea Hens Administered HMG

The effects of HMG administration on the hormonal profile and egg production of the hens are as shown in Table 1. Generally, LH concentration was higher ($P<0.05$) during rainy season (8.71µ/ml) than in the dry season (8.14µ/ml). The post administration period also had higher ($P<0.05$) LH concentration than during the period of administration in guinea hens in each season. The highest concentration of LH was obtained in all the treatments during the second phase of the withdrawal period in the rainy season. However, the different doses administered did not significantly ($P>0.05$) affect the LH concentration but there was significant interaction ($P<0.05$) between season and administration of HMG. LH concentrations for both periods in the dry season were similar (8.207µ/ml and 8.067µ/ml for administration and post administration respectively) but differed ($P<0.05$) from both periods in the rainy season (7.835µ/ml and 9.463µ/ml). In the dry season, HMG administration was better ($P<0.05$) at the 12 IU and 18 IU compared with the Control but concentrations were higher on 6 IU and 18 IU doses. In the rainy season, HMG administration improved LH concentration, except at 6 IU, over the Control with the highest concentration at 24 IU. During the post administration period, all the doses of HMG had better LH concentration than the Control with the highest concentration at dose of 12 IU.

Table 1: Effects of season, efficacy period and dose of HMG on hormonal profile and egg production in guinea hen

Treatments	LH (µ/ml)	FSH (µ/ml)	Hen Day Production (%)	Egg Number
Season				
Dry Season	8.14[b]	5.01[b]	18.91[b]	1.33[b]
Rainy Season	8.71[a]	5.80[a]	28.27[a]	1.98[a]
SEM	0.16	0.19	0.97	0.07
Efficacy Period				
HMG	8.02[b]	5.09[b]	19.18[b]	1.34[b]
Post-HMG	8.82[a]	5.72[a]	28.00[a]	1.97[a]
SEM	0.13	0.13	1.47	0.1
Dose				
0IU	8.30	5.45	28.04[a]	1.96[a]
6IU	8.06	5.29	22.22[a]	1.56[b]
12IU	8.55	5.35	25.52[a]	1.79[a]
18IU	8.76	5.50	14.70[b]	1.05[c]
24IU	8.43	5.43	27.46[a]	1.92[a]
SEM	0.31	0.20	3.17	0.22
Season x Efficacy Period	*	*	*	*
Season x Dose	*	NS	NS	NS
Efficacy Period x Dose	NS	NS	NS	NS
Season x Efficacy Period x Dose	*	NS	NS	NS

Means along each column followed by different superscripts differ significantly (P<0.05)

FSH concentration was also higher (P<0.05) in the rainy season than in the dry season. Concentration was higher (P<0.05) during the period of post administration while doses of HMG did not affect (P>0.05) the concentration of FSH. There was a significant (P<0.05) interaction between season and administration of HMG.

HDP was significantly influenced (P<0.05) by season, administration and dose of HMG. There was better HDP (P<0.05) during the rainy season (28.27%) than in the dry season (18.91%). The post administration period also had better (P<0.05) HDP than the period of administration (28.00% vs. 19.18%). There was significant interaction (P<0.05) between season and efficacy period of HMG on HDP. Irrespective of the doses of HMG administered, HDP was lowest (P<0.05) during the administration period in the dry season (Table 2).

Egg number was significantly (P<0.05) influenced by season, efficacy period of HMG and the dose administered. Administration of 24 IU of HMG resulted in the highest (P<0.05) egg number (1.92) and was similar (1.79) to the 12 IU treatment while administering 18 IU resulted in the lowest number (1.05). The Control had comparable egg number to birds on 24 and 12 IU of HMG. Egg number increased following HMG administration during dry season from 1.33 to 2.35 post administration while in the rainy season, it declined from 1.98 during administration to 1.59 after (Table 2).

Table 2: Interaction between season, efficacy period and dose of HMG on hormonal profile and egg production in guinea hens

Season	Efficacy Period	Dose	LH (µ/ml)	FSH (µ/ml)	Hen Day Production (%)	Average Egg Number
Dry			8.14 ± 0.17	5.01 ± 0.12	18.91 2.72	1.33 ± 0.19
	HMG	**0IU**	8.63 ± 0.31^{abcdef}	5.20 ± 0.48	4.47 ± 1.02	0.31 ± 0.07
		6IU	7.94 ± 0.64^{cdefg}	5.23 ± 0.36	5.47 ± 1.39^{fgh}	0.38 ± 0.10^{fg}
		12IU	8.76 ± 0.69^{abcde}	5.44 ± 0.41	1.43 ± 0.24^{gh}	0.10 ± 0.02^{g}
		18IU	8.95 ± 0.67^{abcde}	5.07 ± 0.41	11.19 ± 2.86^{efgh}	0.78 ± 0.20^{efg}
		24IU	6.76 ± 0.50^{g}	4.52 ± 0.62	0 ± 0^{h}	0 ± 0^{g}
		Mean	8.21 ± 0.29	5.09 ± 0.20^{b}	4.28 ± 1.39^{gh}	0.30 ± 0.10^{fg}
	Post HMG	**0IU**	8.05 ± 0.57^{bcdefg}	5.15 ± 0.32	33.34 ± 3.42	2.35 ± 0.24
		6IU	8.10 ± 0.18^{abcdefg}	4.26 ± 0.31	38.48 ± 7.27^{ab}	2.69 ± 0.51^{ab}
		12IU	7.86 ± 0.54^{defg}	5.09 ± 0.40	30.71 ± 7.32^{abcd}	2.15 ± 0.51^{abcd}
		18IU	8.37 ± 0.64^{abcdefg}	5.12 ± 0.14	37.68 ± 6.84^{ab}	2.64 ± 0.48^{ab}
		24IU	7.95 ± 0.37^{cdefg}	4.99 ± 0.16	17.95 ± 3.94^{cdef}	1.32 ± 0.22^{cdef}
		Mean	8.07 ± 0.20	4.92 ± 0.14^{b}	41.88 ± 9.61^{a}	2.94 ± 0.68^{a}
Rainy Season			8.49 ± 0.21	5.62 ± 0.19	28.27 ± 1.87	1.98± 0.13
	HMG	**0IU**	7.30 ± 0.44^{efg}	5.08 ± 0.44	33.88 ± 2.64	2.37 ± 0.18
		6IU	6.90 ± 0.49^{fg}	4.61 ± 0.69	41.43 ± 6.83^{a}	2.89 ± 0.48^{a}
		12IU	7.74 ± 0.49^{defg}	4.70 ± 0.24	35.00 ± 6.99^{abc}	2.45 ± 0.49^{abc}
		18IU	8.05 ± 0.61^{bcdefg}	5.32 ± 0.31	28.57 ± 6.24^{abcde}	2.00 ± 0.44^{abcd}
		24IU	9.18 ± 0.22^{abcd}	5.71 ± 0.43	26.33 ± 4.83^{abcde}	1.85 ± 0.33^{abcde}
		Mean	7.83 ± 0.25	5.08 ± 0.20^{b}	38.09 ± 3.30^{ab}	2.67 ± 0.23^{ab}
	Post HMG	**0IU**	9.23 ± 0.39^{abcd}	6.35 ± 0.45	22.65 ± 2.17	1.59 ± 0.15
		6IU	9.30 ± 0.19^{abcd}	7.07 ± 0.68	26.78 ± 5.27^{abcde}	1.88 ± 0.37^{abcde}
		12IU	9.85 ± 0.55^{a}	6.17 ± 0.49	21.72 ± 7.29^{bcde}	1.56 ± 0.49^{bcde}
		18IU	9.67 ± 0.60^{abc}	6.50 ± 0.90	24.64 ± 1.81^{abcde}	1.73 ± 0.13^{abcde}
		24IU	9.83 ± 0.04^{ab}	6.52 ± 0.49	14.52 ± 2.49^{defgh}	1.02 ± 0.17^{defg}
		Mean	9.58 ± 0.17	6.52 ± 0.25^{a}	25.59 ± 5.13^{abcde}	1.79 ± 0.36^{abcde}
		SEM	0.87	0.56	2.36	0.17
Grand Total			8.29 ± 0.14	5.28 ± 0.11	23.59 ± 1.71	1.66 ± 0.82

Means along each column followed by different superscripts differ significantly (P<0.05)

Correlated Response between Hormone Concentrations and Egg Production

LH and FSH levels were positively correlated in both seasons but the correlation was highly significant (P<0.01) only in the rainy season (Table 3). FSH was positively and lowly correlated (P>0.05) with egg production in both seasons. Low negative correlation existed between egg production and LH in the dry season while positive and non-significant (P>0.05) correlation was observed in the rainy season.

LH and FSH were positively correlated with each other during the periods of HMG administration and post HMG administration (Table 4). However, the correlation was highly significant (P<0.01) in the post HMG administration period (0.448). Egg production was positively correlated with FSH during the HMG administration period (0.105) but negatively correlated during the post HMG period (-0.276). Egg production and LH were negatively correlated in both administration periods in the two seasons with significance

(P<0.05) during the post administration period only (-.0.359).

LH and FSH were positively but non-significantly correlated with each other at the various doses of HMG (Table 5). Correlation between monthly hormone levels and egg production were low and non-significant (P>0.05). FSH was positively correlated with egg production at doses of 0 and 24 IU while there were negative correlations at other doses. LH was negatively correlated with egg production at doses of 0, 12 and 18 IU but positively correlated at 6 and 24 IU though not significantly (P>0.05).

Table 3: Effect of Season on Pearson's Bivariate Correlation between Hormones and Egg Production in Guinea Hens

	Trait 2				
Trait 1	**FSH (µ/ml)**	**LH (µ/ml)**	**TEN**	**HDP(%)**	**Season**
FSH (µ/ml)					Dry Season
					Rainy season
LH (µ/ml)	.148				Dry Season
	.422**				Rainy season
TEN	.016	-.096			Dry Season
	.170	.244			Rainy season
HDP	.049	-.100	.992**		Dry Season
	-.182	-.201	.674**		Rainy season

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

TEN: Total Egg Number

Table 4: Effect of efficacy period of HMG on Pearson's bivariate correlation between hormones and egg production

	Trait 2				
Trait 1	**FSH (µ/ml)**	**LH (µ/ml)**	**TEN**	**HDP (%)**	**Efficacy Period**
FSH (µ/ml)					HMG
					Post HMG
LH (µ/ml)	.168				HMG
	.448**				Post HMG
TEN	.105	-.114			HMG
	-.276	-.359*			Post HMG
HDP	.105	-.114	1.000**		HMG
	-.174	-.251	.975**		Post HMG

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

Table 5: Effect of doses of HMG on Pearson's bivariate correlation between Hormones and egg production

Trait 1	Trait 2 FSH (µ/ml)	LH (µ/ml)	TEN	HDP (%)	Dose (IU)
FSH (µ/ml)					0
					6
					12
					18
					24
LH (µ/ml)	.327				0
	.323				6
	.249				12
	.286				18
	.404				24
TEN	.189	-.096			0
	-.116	.036			6
	-.029	-.276			12
	-.018	-.022			18
	.085	.260			24
HDP (%)	.193	-.357	.797**		0
	-.146	-.254	.829**		6
	-.138	-.304	.852**		12
	.008	-.059	.776**		18
	.269	.458*	.862**		24

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

Table 6: Pearson's Bivariate Correlation between Hormones and Egg Production in Guinea Hens

Trait 1	Trait 2 FSH (µ/ml)	LH (µ/ml)	TEN	HDP	Group
FSH (µ/ml)					All
LH (µ/ml)	.322**				All
TEN	.019	-.026			All
HDP (%)	.039	-.089	.835**		All

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Over the six months observation period, there was significant positive correlation between LH and FSH concentration (Table 6) but the correlations between the hormone levels and egg production over the experimental period were low and not significant. However while FSH and TEN were positively correlated, LH and TEN were negatively correlated.

Effect of HMG Administration on Reproductive Tract Size

The effect of HMG administration on the

reproductive tract of the guinea hen is shown in Table 7. The different levels of HMG administration had no significant (P>0.05) effect on the ovary weight, oviduct length and the number of developing follicles. Ovary weight was lowest, 32.02g, in birds administered 12 IU and highest, 48.41g, in the Control group. The length of the oviduct (33.37cm) was shortest in birds administered 24 IU and longest (43.03cm) in birds administered 18 IU of HMG. Except for the Control group, there were higher numbers of large white follicles than large and small yellow follicles in the groups administered HMG. However, there were more (P>0.05) small yellow follicles in the Control group than in the hens administered HMG. The Control group had the least (P>0.05) number of large white follicles compared with the groups administered HMG.

Table 7: Effect of HMG administration on reproductive tract of guinea hens

Doses	Ovary wt (g)	Oviduct length (cm)	No of large yellow follicles (>8mm)	No of small yellow follicles (2-8mm)	No of large white follicles (2-5mm)
0IU	48.41	39.97	4.67	8.67	7.37
6IU	43.44	40.57	4.33	3.33	9.00
12IU	32.02	34.15	4.50	4.25	10.00
18IU	46.37	43.03	4.00	5.33	10.67
24IU	36.36	33.37	3.33	5.67	9.33
SEM	10.63	3.18	0.57	1.11	1.05

Discussion

Both LH and FSH levels were elevated in laying guinea hens used in this study and differed with season and HMG administration. Although the actual cause of the elevated values could not be ascertained, egg production in poultry is generally controlled by the rate of growth and differentiation of ovarian follicles that are controlled by hormones and other physiological conditions (11). Thus the differences in egg laying performance according to season and HMG administration could be associated with differences in plasma levels of reproductive hormones such as FSH and LH (12, 13, 14, 15). There have been reports on the influence of age, feeding and photoperiod on reproductive hormones for egg-type layers and broiler breeders (16, 17, 18, 19, 14, 15, 20). Thus the differences in seasonal levels of the hormones in the present study can be attributed to environmental or climatic factors especially photoperiod and humidity. Photoperiod has been reported as essential in manipulating sexual maturity and reproductive performance in avian species as upon photostimulation, GnRH stimulates the release of gonadotropins (FSH and LH) from the anterior pituitary gland, which in turn triggers gonadal development and the synthesis of steroid hormones (21, 22). Both environmental temperature and relative humidity interact to affect severity of heat stress on animals, when, relative humidity is high and ambient temperature is also high, then evaporative heat loss is reduced (23, 24, 25). The level of egg production was thus high in the rainy season when plasma concentration of both LH and FSH were found to be high.

The positive correlations between concentrations of the hormones and egg production confirm previous report (11) but contrary to others (20) that there was no correlation between plasma FSH and LH and egg production. However, the low correlations between egg production and LH as well as FSH might indicate lack of real association between these hormones and egg production. This is similar to the negative and low correlation between FSH and egg production at both pre and post peak egg production and negative correlation between LH and egg production at pre peak production (11).

Egg number was significantly higher in the post administration period in this study and this might be as a result of the time interval required for the exogenous hormone to take effect. Thus the time required for HMG to stimulate egg production became attained during the post administration period, which was about eight weeks from beginning of administration. As the effect of HMG administered wore off post administration, egg production reduced. It has been reported that changes in reproductive hormone secretion represent the final sequence in the neuroendocrine pathway (26). It appears therefore that constant or at least periodic administration of HMG might be required to sustain egg production in the guinea fowl. The doses of the HMG administered did not result in significant increase in egg number over the Control and there was no particular trend in the groups administered HMG. The egg number obtained in this study was significantly higher in the rainy than dry season. This is in line with previous reports (9, 27, 28, 29, 30, 31) that more eggs are produced in the rainy season. This they attributed to either abundance of feed in the wild during the rainy season or a more conducive environment that can influence production of FSH and LH.

Results obtained in this study for the harvested ova does not agree with the report of (32) who administered 75 IU of HMG to waterfowls for ten days and reported that the ovarian size, number of differentiating oocytes (vitellogenic and post-vitellogenic) and theca layer diameter were significantly improved compared with the control group while the number of undifferentiated and pre-vitellogenic oocytes, nucleus and arteriole diameter were similar between the control and experimental groups. While they concluded that HMG has a positive and meaningful effect on ovarian follicular recruitment, the different categories of ova in this study were not significantly different although the number of large white follicles was higher with the doses of HMG up to 18 IU. Thus the number of immature and ripe ova was not significantly influenced by HMG administration in this study.

Conclusions and Applications

Egg production in guinea hens especially during dry season can be improved through administration of HMG.

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