

Full Length Research Paper

Effect of testosterone and growth hormone injection before puberty on follicles size, rate of egg production and egg characteristics of the Mazandaran Native breeder hens

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Accepted 21 July, 2008

Egg shell quality and egg internal quality are of major importance to the egg industry worldwide. This experiment was conducted to evaluate the effect of testosterone and growth hormone (hGH) on egg production and characteristics. The aim of this trial is to test this hypothesis that one injection of these two hormones before puberty has any effects on egg production of Mazandaran native breeder hens. Two hundred native pullet used in a completely randomized design with 4 treatments, 5 replicates and 10 hens in each box. Hens were fed on common diet and hormones injected subcutaneously based on body weight (BW). The four treatments were: 1) injection of hGH (100 µg/kg BW), 2) injection of testosterone (500 µg/kg BW), 3) injection of hGH (100 µg/kg BW) + injection of testosterone (500 µg/kg BW) and 4) injection of 100 µl distilled water (control). Injection was before 5% oviposition of flock (flock puberty). Egg production and characteristics were determined. Treatments affected ovary weight, small white, large white and small yellow follicles and some egg parameters. GH and testosterone can alter follicles size in ovary, and it is concluded that GH+testosterone injection is more effective on small and large follicles.

Key words: Testosterone, growth hormone, follicle size, egg production, breeder hen.

INTRODUCTION

Egg quality may be measured as egg weights (EWT), shell breaking strength (SBS), egg length (EL), egg width (EWD), egg shape index (ESI) and egg score (ES). After measuring the external characters, the eggs were broken open on the egg breaking stand for measuring their height of shell thickness (ST), albumen height (AH) and Haugh unit (HU). Indigenous poultry birds are well adapted to harsh environment and they produce eggs and meat at least possible cost. The birds require no extra care and housing which makes them suitable for backyard poultry farming. Evaluation of the external and inter-

nal quality of chicken eggs is important because of consumer preferences for better quality eggs. It is generally agreed that all characteristics of egg quality have a genetic basis. Egg quality has been defined by Stadelman (1977) as the characteristics of an egg that affect its acceptability to the consumers. Egg quality is the most important price contributing factor in table and hatching eggs. Therefore, the economic success of a laying flock solely depends on the total number of quality eggs produced. Quality of chicken eggs may vary due to several factors like rearing, temperature, relative humidity and season. One of factors that affect egg production and egg quality is hormonal status of hen. Testosterone and growth hormone (via IGFs system) can be effective in egg production. The ovary of the hen secretes estrogen (estradiol and estrone), progesterin (progesterone), and

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androgen [testosterone (T) and dihydrotestosterone (DHT)] (Kawashima et al., 1999). There is little evidence supporting the role of androgen in the reproductive physiology of the hen. The action of the ovarian hormones is thought to be manifested by binding to their receptors in target tissues (Jensen, 1990).

In the domestic hen, an increase in plasma progesterone, originating from the mature and maturing preovulatory ovarian follicles induces a preovulatory release of luteinizing hormone (LH) (Etches, 1996), by stimulating the release of gonadotrophin-releasing hormone (Fraser and Sharp, 1978). The increase in plasma progesterone and LH 3–6 h before ovulation is preceded by increased plasma testosterone and plasma estradiol (Etches and Cheng, 1981). Estradiol does not participate directly in the positive feedback control of LH release, but it is necessary to prime the hypothalamus to allow the positive feedback action of progesterone. A role for the preovulatory release of testosterone in the ovulatory process is suggested by the finding that injection of testosterone in laying hens with mature preovulatory follicles induces ovulation (Croze and Etches, 1980) and a preovulatory-like release of LH, while blockage of testosterone action by passive immunization or active immunization against testosterone (Rangel et al., 2005) blocks ovulation. Further, active immunization against testosterone induces atresia of preovulatory yellow yolky follicles, but does not prevent their development (Rangel et al., 2005), while chronic treatment with the steroidal androgen receptor antagonist, cyproterone acetate, blocks ovulation and induces ovarian regression (Luck, 1982). While injection of hens with mature preovulatory follicles with progesterone induced ovulation within 8 h, injection with testosterone induced ovulation after more than 9 h, suggesting that testosterone must first be converted to an “active substance” before ovulation could be induced. Croze and Etches (1980) found that ovulation could only be induced using doses of testosterone which produced unphysiologically high plasma concentrations, and suggested that the preovulatory release of testosterone has “a preparatory or priming action on the hypothalamo–pituitary–ovarian system which facilitates the preovulatory release of LH”. Blocking the action of the preovulatory surge of testosterone, with its specific antagonist flutamide (a non-steroidal androgen receptor antagonist; Mainwaring et al., 1987), will halt the predicted oviposition and the preovulatory surges of plasma testosterone, progesterone, estradiol and LH in the laying hen. Rangel et al. (2006) demonstrated that in the domestic hen, acute blockage of testosterone action during the ovulatory cycle, by the inhibition of its specific receptor with flutamide, blocks egg laying and the associated preovulatory surges of progesterone, estradiol and LH. Earlier studies suggested that testosterone must first be converted to an “active substance” before it can induce ovulation (Fraps, 1955) or act to prime the Hypothalamo–pituitary–ovarian system to facilitate the preovu-

latory release of LH (Croze and Etches, 1980). The possibility that testosterone must be first converted to an “active substance” to exert a direct stimulatory effect on LH release is unlikely since all evidence points to progesterone being the principal steroid directly inducing the preovulatory release of LH (Johnson and van Tienhoven, 1980) and progesterone is not a metabolite of testosterone (Norman and Litwack, 1997). The possibility that testosterone primes the hypothalamo–pituitary–ovarian system to facilitate the preovulatory release of LH therefore merits closer analysis. A combination of estrogen and progesterone treatment primes the hypothalamo–pituitary system of the ovariectomised hen to make it responsive to the stimulatory action of progesterone on LH release (Wilson and Sharp, 1975). It has not been established whether testosterone might mimic the priming effect of estrogen. However, it seems unlikely that the preovulatory increase in plasma testosterone is solely responsible for priming the hypothalamo–pituitary system for the stimulatory action of progesterone on LH release since the base-line plasma concentrations of estrogen in the flutamide-treated hens were not depressed and should have been adequate to exert a priming effect on the hypothalamo–pituitary system (Rangel et al., 2006). It is therefore possible that the preovulatory peak of testosterone may act to prime the ovary to facilitate the preovulatory release of progesterone. The principal ovarian source of progesterone for the preovulatory surge is the granulosa cell layer of the mature preovulatory follicle, with subsidiary contributions from the granulosa layer of the next most mature preovulatory follicle (Bahr et al., 1983). These granulosa cells are targets for testosterone since they contain nuclear androgen receptors (Yoshimura et al., 1993).

Ovulations and ovipositions cease in the arrested laying hens, but the entrance of follicles into the follicular hierarchy and hierarchical growth continues, leading to an accumulation of numerous mature follicles in the ovary. This study will increase our understanding of the functional significance of the preovulatory surge of testosterone. The present study was performed to obtain evidence for the direct action of one injection of testosterone and growth hormone, before puberty, on the egg production and its characteristics in Mazandaran native breeder hens.

MATERIALS AND METHODS

Two hundred native pullet used in a completely randomized design with 4 treatments, 5 replicates and 10 hens in each box at the center of native hen breeding in Mazandaran province in north of Iran (Center of native hen breeding). A commercial diet was fed during the experiment (20% crude protein; 2800 Cal/kg diet and 100 g/d/hen). Birds were maintained under day-light (about 12 h), and had free access to water at all times. All birds used in the study were from the same hatch. Hens were fed on common diet and hormones were injected subcutaneously based on body weight

Table 1. Effect of GH and testosterone on ovary weight, oviduct length and follicles diameter, two weeks after injection.

Parameter	Treatment*				SEM**
	Control	GH	Testosterone	GH + Testosterone	
OYW	33.276 ^{ab}	33.532 ^{ab}	32.282 ^b	37.018 ^a	0.610
OTL	50.740 ^a	54.340 ^a	51.700 ^a	54.260 ^a	1.043
SWF	0.436 ^b	0.558 ^a	0.548 ^a	0.586 ^a	0.014
LWF	0.530 ^b	0.658 ^{ab}	0.608 ^{ab}	0.696 ^a	0.020
SYF	0.694 ^b	0.828 ^{ab}	0.788 ^{ab}	0.936 ^a	0.026
LYF	1.022 ^a	1.162 ^a	1.192 ^a	1.27 ^a	0.052
F5	1.464 ^a	1.546 ^a	1.628 ^a	1.674 ^a	0.037
F4	1.882 ^a	1.952 ^a	1.904 ^a	2.090 ^a	0.046
F3	2.324 ^a	2.224 ^a	2.236 ^a	2.358 ^a	0.033
F2	2.494 ^a	2.524 ^a	2.520 ^a	2.582 ^a	0.015
F1	2.638 ^a	2.638 ^a	2.700 ^a	2.792 ^a	0.024

*100 µg/kg BW, hGH, 500 µg/kg BW, testosterone and 100 µl distilled water (Control) was used.

**Standard error of mean.

OTL: Oviduct Length; OYW: Ovary Weight; SWF: Small White Follicle; LWF: Large White Follicle; SYF: Small Yellow Follicle; LYF: Large Yellow Follicle; F5 to F1: Follicles f5 to f1.

Within each row, treatments that carry the same superscript letter are not significantly different at $P < 0.05$ (DMRT).

(BW). The four treatments were: 1) injection of hGH (100 µg/kg BW), 2) injection of testosterone (500 µg/kg BW), 3) injection of hGH (100 µg/kg BW) + injection of testosterone (500 µg/kg BW) and 4) injection of 100 µl distilled water (Control). Injection was before 5% oviposition of flock (flock puberty). Two weeks after injection, all eggs collected from each box and were studied for various egg quality traits. The characters of eggs were measured including egg weights (EWt), egg shell difficulty (ESD), egg length (EL), egg width (EWd), egg shape index (ESI) and egg score (ES). After measuring the external characters, the eggs were broken open on the egg breaking stand for measuring their height of egg shell thickness (ESTs), albumen height (AH) and Haugh unit (HU). Haugh units were calculated from the height (H) of the albumen and the egg weight using the simplified Haugh unit formula (Eisen et al., 1962):

$$HU = 100 \log (H - 1.7W^{0.37} + 7.57)$$

The eggs produced under each box stored at 12°C and were weighed every week during experimental period. Shape index was calculated by using egg shape factor meter:

$$\text{Egg Shape Index (ESI)} = \frac{\text{Horizontal diameter (width)}}{\text{Vertical diameter (length)}} \times 100$$

Yolk index was calculated by using Vernier Calipers:

$$\text{Yolk Index (YI)} = \frac{\text{Yolk height}}{\text{Yolk diameter}} \times 100$$

Average for the whole egg laying period of hen-day percent lay was worked out and termed as percent lay. Hen-day egg production on daily basis was calculated by adopting the following formula given by North (1984);

$$\text{HDP} = \frac{\text{Number of eggs produced on daily basis}}{\text{Number of birds available in the flock on that day}}$$

Hen-day egg production for the whole period was worked out by summing up the daily hen-day egg production of the flock.

Measures for the eggs from four treatments of hens at two weeks after injection were compared using the General Linear Models program of SAS 8.02 (SAS, 2001). The Duncan's multiple range tests was used to compare the means. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Results are shown in Tables 1 to 4. GH is usually, but not always, required for the timing of sexual maturation, since delayed or absent puberty is often associated with GH-deficient or GH-resistant states and GH administration accelerates puberty (Hull and Harvey, 2001). GH may play a particularly important role in early, follicle-stimulating hormone (FSH)-independent activities, since GH-binding activity peaks during early folliculogenesis in porcine follicles (Quesnel, 1999) and fish ovarian homogenates (Gomez et al., 1998). Indeed, *in vivo* and *in vitro* studies suggest that GH stimulates growth and prevents atresia in small follicles. For instance, GH administration *in vivo* increases the number of small follicles in cattle (Gong et al., 1991, 1993) and horses (Cochran et al., 1999).

Increase of ovary weight in GH + testosterone treatment was not significant, but was significant in testosterone treatment. Testosterone can stimulate hypertrophy of cells in ovary. The diameter of small white follicles was significantly increased in GH, testosterone and GH + testosterone treatments compared with control. The diameter of large white and small yellow follicles was significantly increased by treatment of GH + Testosterone (Table 1). This effect is important and it may be because

Table 2. Effect of GH and testosterone on egg parameters at the first week after injection.

Parameter	Treatment*				SEM**
	Control	GH	Testosterone	GH + Testosterone	
EWT	41.451 ^a	36.933 ^a	40.125 ^a	43.238 ^a	1.036
ST	0.371 ^a	0.341 ^a	0.376 ^a	0.352 ^a	0.010
SBS	1.139 ^a	0.905 ^a	1.523 ^a	0.947 ^a	0.113
EWD	4.022 ^a	3.673 ^b	3.713 ^b	3.800 ^b	0.028
EL	5.022 ^a	4.767 ^b	5.028 ^a	5.189 ^a	0.028
ESI	79.982 ^a	72.772 ^a	76.005 ^a	76.429 ^a	1.659
ES	4.267 ^b	4.140 ^b	4.535 ^{ab}	5.011 ^a	0.080
AH	8.082 ^a	8.270 ^a	7.085 ^b	8.020 ^{ab}	0.155
HU	94.568 ^{ab}	96.889 ^a	89.725 ^b	93.808 ^{ab}	0.824

*100 µg/kg BW, hGH, 500 µg/kg BW, testosterone and 100 µl distilled water (Control) was used.

**Standard error of mean.

EWT: Egg Weight; ST: Shell Thickness; SBS: Shell Breaking Strength; EWD: Egg Width; EL: Egg Length; ESI: Egg Shell Index; ES: Egg Score; AH: Albumen Height; HU: Haugh Unit.

Within each row, treatments that carry the same superscript letter are not significantly different at $P < 0.05$ (DMRT).

Table 3. Effect of GH and testosterone on egg parameters at the second week after injection.

Parameter	Treatment*				SEM**
	Control	GH	Testosterone	GH + Testosterone	
EWT	39.952 ^a	41.670 ^a	40.063 ^a	40.301 ^a	0.421
ST	0.340 ^{ab}	0.344 ^{ab}	0.354 ^a	0.331 ^b	0.002
SBS	0.947 ^a	0.883 ^a	0.838 ^a	0.857 ^a	0.058
EWD	3.777 ^{ab}	3.813 ^a	3.747 ^b	3.763 ^{ab}	0.009
EL	4.951 ^a	4.978 ^a	4.923 ^a	4.989 ^a	0.021
ESI	76.395 ^a	76.752 ^a	76.840 ^a	75.696 ^a	0.405
ES	4.177 ^a	4.094 ^a	4.206 ^a	4.300 ^a	0.058
AH	8.310 ^b	8.892 ^a	8.437 ^{ab}	8.696 ^{ab}	0.084
HU	96.273 ^a	98.434 ^a	96.859 ^a	97.991 ^a	0.391

*100 µg/kg BW, hGH, 500 µg/kg BW, testosterone and 100 µl distilled water (Control) was used.

**Standard error of mean.

EWT: Egg Weight; ST: Shell Thickness; SBS: Shell Breaking Strength; EWD: Egg Width; EL: Egg Length; ESI: Egg Shell Index; ES: Egg Score; AH: Albumen Height; HU: Haugh Unit.

Within each row, treatments that carry the same superscript letter are not significantly different at $P < 0.05$ (DMRT).

of increase in growth factors, especially IGFs, in small and large growing follicles. Also GH can increase yolk production in liver leading to increased egg production.

At the first week after injection, egg length in GH treatment and egg width in GH, testosterone and GH + testosterone were significantly reduced compared with control. Egg score was significantly increased in GH + testosterone treatment, but albumen height and Haugh unit reducing in testosterone treatment was significant (Table 2).

At the second week after injection, shell thickness and egg width were significantly reduced in GH + testosterone and testosterone treatment, respectively. Increase of albumen height in GH treatment was significant (Table 3). There was no significant change in hen-day egg production (HDP) three weeks after injection (Table 4).

The role of androgens in the neuroendocrine control of follicular function is enigmatic (Etches, 1990). GH may be implicated in the control of reproduction in birds as well. In laying hens, the GH and GH-receptor genotypes have been found to be associated with age at first egg and the rate of egg production (Lebedeva et al., 2004). Williams et al. (1992) reported that the number of small follicles in the domestic hen rises after treatment with ovine GH. In turkeys, higher plasma GH concentrations and pituitary GH mRNA expression were detected in egg-laying than in nonlaying hens. Differences in egg production between *ad libitum* and restricted fed broiler have been demonstrated to be related to changes in the GH/IGF-I axis (Lebedeva et al., 2004).

The complex nature of the process of formation of the internal components of the egg and the egg shell mean

Table 4. Hen-day egg production (HDP) at three weeks after injection of GH and testosterone.

Parameter	Treatment*				SEM**
	Control	GH	Testosterone	GH + Testosterone	
1 st Week	0.322 ^a	0.398 ^a	0.511 ^a	0.244 ^a	0.054
2 nd Week	0.994 ^a	0.941 ^a	1.089 ^a	0.805 ^a	0.098
3 rd Week	1.664 ^a	1.580 ^a	1.825 ^a	1.332 ^a	0.116

*100 µg/kg BW, hGH, 500 µg/kg BW, testosterone and 100 µl distilled water (Control) was used.

**Standard error of mean.

Within each row, treatments that carry the same superscript letter are not significantly different at $P < 0.05$ (DMRT).

that quality problems may arise at any of several stages during the formation of the egg. Also, problems with egg internal quality and egg shell quality may result from a combination of factors, rather than from a single factor (Robert, 2004). GH can increase yolk production in liver by stimulating IGF systems, but further injection may be needed. On the other hand it may stimulate other growth factors in small follicles. Other investigations are needed to examine further injections or infusion of GH with testosterone injection to get more evidence about this mechanism.

ACKNOWLEDGEMENTS

The authors were grateful to the University of Tehran for providing the financial support to carry out this research work and also the Ministry of Jihad-E-Agriculture of Mazandaran. We thank Eng. Talebi, Eng. Kuhi and other employers for their kind attention during this investigation.

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