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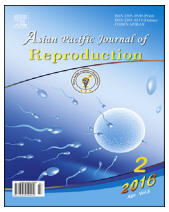

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Effects of intramuscular injections of vitamin E-selenium and a gonadotropin releasing hormone analogue (GnRHa) on reproductive performance and blood metabolites of post-molt male broiler breeders

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ABSTRACT

Objective: To investigate the effects of intramuscular injection of vitamin E-selenium and a GnRH analogue (GnRHa) on reproductive performance and serum biochemical parameters in post-molt male broiler breeders.

Methods: A total of 32 ROSS 308 male broiler breeders (60 weeks of age) were induced to molt and then were randomly distributed into four groups: group 1 (control) without any injection, group 2 subjected to intramuscular of 0.1 mL/kg body weight of vitamin E-selenium, group 3 subjected to intramuscular of 0.3 mL. Receptal solution as a GnRHa and group IV subjected to intramuscular both of vitamin E-selenium and GnRHa.

Results: The results showed that the egg hatchability and fertility percentages were increased and the eggs infertility percentage declined significantly in groups 3 and 4 ($P < 0.05$). However, eggs and chicks weight was not affected by experimental treatments ($P > 0.05$). The results of blood biochemical parameters indicated that serum glucose was higher in group 3 which was injected with GnRHa ($P < 0.05$). The experimental treatments did not alter hepatic enzymes activity including AST and ALT ($P > 0.05$). The results showed that serum testosterone concentration was increased in groups 3 and 4 ($P < 0.05$). In addition, the serum concentration of T4 (tetraiodothyronie) was higher in groups 3 and 4 ($P < 0.05$). However, the T3 (triiodothyronine) concentration was not influenced by experimental treatments ($P > 0.05$).

Conclusions: It is concluded that reproductive performance and serum testosterone in post-molt male broiler breeders were improved by treated with vitamin E-selenium and GnRHa.

1. Introduction

Age has an adverse effect on the reproductive success in birds. Fertility of domestic roosters kept under controlled conditions, peaks at about 37 weeks of age and then decreases rapidly at 45 weeks of age. The low-fertility roosters are characterized by: (1) a significant decline in concentration of ejaculated sperm [1]; (2) malformations of sertoli cell ectoplasmic specializations [2]; and (3) structural changes in the Leyding cells [3]. Force molting is an economical approach which improves the productive and reproductive life span of aged roosters. However, it has been previously reported that semen quality and quantity in molted males is affected by the process of molting [4]. Recently, it was shown that semen quality in molted male broiler breeders was improved when they were fed with different feed additives in the post-molt period [5]. Several reports have been published on the beneficial effects of Vitamin E on improving reproductive traits in male poultry [6–8]. However, most of these studies have been conducted on the male birds at the peak of production. Moreover, the effects of vitamin E were examined without consideration of other factors such as selenium. Selenium and vitamin E are involved in many biochemical and physiological processes in animal organism, including those related to reproduction [9]. Particularly relevant to semen quality is the antioxidant enzyme glutathione peroxidase (GSH-Px), a selenium dependent enzyme that serves to protect cellular membranes from peroxidative damages [10]. This enzyme has an important role in the maintenance of testicular

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function, spermatogenesis and spermatozoa functions [11]. Therefore, in the present experiment, we used a supplement in term of Selenovit premix containing vitamin E and selenium.

A number of hormones control sexual maturity, semen production, and the behavior connected with reproduction in male broiler breeders. In this regard, testosterone is the primary sex steroid in the avian testis. So, relative changes in the secretion of this hormone are likely to correlate with, or be a reliable index of, testicular activity [12]. Also, it is well known that testicular function in birds and human is controlled by gonadotropin releasing hormones (GnRH) secretion which is responsible of stimulating the gonadotropes of the pituitary to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) [13]. Therefore, the administration of a synthetic GnRH may be resulted in the sustained release of LH from the anterior pituitary and the production Leydig cell enzymes capable of converting cholesterol into testosterone in post-molted male broiler breeders.

The objectives of this study were to investigate the effects of vitamin E and a GnRHa (Receptal solution) on reproductive traits and some blood biochemical parameters of post-molted male broiler breeders.

2. Materials and methods

2.1. Birds and treatments

This experiment was performed in a commercial broiler breeder farm. All procedures followed in this experiment were approved by Islamic Azad University, Qaemshahr branch, Qaemshahr, Iran. Thirty two ROSS-308 male broiler breeders at the age of 60 weeks were induced to molt with ZnO at the rate of 3000 mg/kg of feed with a moderate decrease in lighting schedule from 16 to 12 h and they were offered 50 g/bird feed on the daily basis [5]. After completion of molting roosters were randomly assigned to four groups with four replicates (pens) per group in a completely randomized design. Each pen was equipped with a drinker, two separate-sex feeders, and a nest box. The birds were reared on floor pens and average temperature of day and night was 24 °C.

Treatment groups were as following: group I: male broiler breeders without any injection; group II: male broiler breeders were injected with 0.1 mL/kg body weight of vitamin E-selenium; group III: male broiler breeders were injected with receptal solution (0.2 mL) as a GnRHa; and group IV: male broiler breeders were injected with both of vitamin E-Selenium and GnRHa. Receptal solution used in this experiment is a GnRH analogue with a chemical entity of Buserelin acetate (0.0042 mg/mL). This solution was injected to male broiler breeder of each pen, weekly. The injectable solution of vitamin E-Selenium was purchased from Makian Daru Company (Iran). This solution contained 50 and 0.5 mg/mL of vitamin E and Sodium Selenite, respectively.

2.2. Reproductive traits

In order to study reproductive traits in this experiment, eight hens at the age of 40 weeks were subjected to each pen. The separate-sex feeding was applied for hens. Therefore, the hens did not access to male broiler breeders diets. The reproductive data including hatchability, fertility, infertility, egg weight and chick weight were recorded during eight weeks. Eggs were collected and weighted daily and incubated for 10 days. Then, the eggs were examined for fertility. The fertile eggs were subjected to hatchery machine and hatchability percentage was measured at the end of hatchery time. In addition, the weight of hatched chicks was recorded. The overall means of these parameters for eight weeks were calculated and statistically analyzed.

2.3. Blood parameters

Heparinized blood samples (5 mL) were taken from each male broiler breeder via the brachial wing vein at the end of the experiment. The blood sample was drawn and allowed to clot at room temperature (18 °C) for 2 h prior to serum collection. Serum was separated by centrifugation and stored at −20 °C for further analysis. A part of blood sample was prepared to measure the serum glucose, cholesterol and total lipids concentrations using commercial diagnostic kits (Pars Azmon, Tehran, Iran). Also, sera samples were used to measure the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as the indicators of liver health. The liver enzymes activity was assayed by auto analyzer (ALCYON 300). The concentration of triiodothyronine (T_3) and thyroxine (T_4) were determined using kits which provided from Tabeshyarnoor Company in Iran. Serum testosterone was also assayed by ELISA procedure described by Sauer MJ [14].

2.4. Statistical analysis

Data were subjected to ANOVA in a completely randomized design with four treatments and statistically analyzed using SAS (v. 9.1, SAS Inst. Inc., Cary, NC, USA). Pen was the experimental unit. Statistical significance of differences among treatments was done using the Duncan's multiple range tests at ($P < 0.05$).

3. Results

The results of reproductive performance of post-molt male broiler breeders in response to experimental treatments are shown in Table 1. The weight of eggs and chicks were not influenced by treatments. The results indicated that infertile eggs

Table 1
Effects of treatments on reproductive traits of male broiler breeders.

Treatments	Infertile eggs (%)	Fertile eggs (%)	Hatchability (%)	Egg weight (g)	Chick weight (g)
Control	22.89 ± 2.50^a	77.05 ± 4.30^c	68.42 ± 4.20^c	63.32 ± 2.20	44.47 ± 5.70
Vit E-Sel	15.78 ± 1.20^b	84.57 ± 4.50^b	73.85 ± 4.30^b	61.74 ± 2.10	44.36 ± 5.80
Receptal	12.29 ± 1.30^c	87.66 ± 4.60^a	79.47 ± 4.40^a	62.34 ± 2.00	44.51 ± 4.90
Vit E-Sel + Receptal	11.45 ± 1.40^c	88.54 ± 4.80^a	80.43 ± 4.50^a	62.21 ± 2.40	44.46 ± 4.80

Means without a common superscripts in per column are significantly different ($P < 0.05$).

Table 2
Effects of treatments on blood biochemical parameters in male broiler breeders (mg/L).

Treatments	Cholesterol	Total lipids	Glucose	Calcium
Control	98.00 ± 8.90	466.25 ± 22.40	219.00 ± 12.10^{c}	176.75 ± 14.10
Vit E-Sel	100.75 ± 7.70	469.75 ± 31.00	226.50 ± 14.70^{bc}	178.75 ± 13.90
Receptal	96.25 ± 8.80	471.76 ± 26.80	235.55 ± 11.90^{a}	175.55 ± 10.30
Vit E-Sel + Receptal	99.50 ± 7.90	470.51 ± 24.70	233.00 ± 9.90^{ab}	178.00 ± 11.40

Means without a common superscripts in per column are significantly different ($P < 0.05$).

Table 3
Effects dietary treatments on blood hormones and liver enzymes in male broiler breeders.

Treatments	T4 (μg/mL)	T3 (ng/mL)	Testosterone (ng/mL)	AST (U/L)	ALT (U/L)
Control	5.77 ± 0.30^{b}	3.97 ± 0.20	1.74 ± 0.07^{c}	36.89 ± 4.30	8.36 ± 1.10
Vit E-Sel	5.85 ± 0.30^{b}	3.75 ± 0.30	1.82 ± 0.04^{bc}	35.83 ± 3.90	7.54 ± 1.70
Receptal	6.21 ± 0.60^{ab}	4.02 ± 0.20	1.79 ± 0.06^{ab}	37.68 ± 3.80	8.60 ± 1.50
Vit E-Sel + Receptal	6.32 ± 0.50^{a}	4.18 ± 0.40	1.93 ± 0.05^{a}	36.18 ± 4.00	7.86 ± 1.40

Means without a common superscripts in per column are significantly different ($P < 0.05$).

(%) were greater in control group (treatment I) compared with other groups ($P < 0.05$). In this regard, the percentage of fertile eggs and hatchability was significantly increased in treatment III and IV ($P < 0.05$).

Effects of experimental treatments on blood biochemical parameters are presented in Table 2. These results showed that the serum concentration of cholesterol, total lipids and calcium did not alter by treatments. However, the glucose level was affected by experimental treatments ($P < 0.05$). The male birds treated by GnRHa (III) and control group (I) had the greatest and lowest serum glucose level, respectively.

The means of blood hormones including T3, T4 and testosterone with serum concentration of liver enzymes (ALT and AST) are shown in Table 3. Results showed that the liver enzymes activities and T3 concentration were not influenced by experimental treatments. In contrast, the serum concentration of T4 and testosterone altered by treatments ($P < 0.05$). In this regard, the levels of T4 and testosterone were higher in male birds of treatment IV.

4. Discussion

The reproductive traits of post-molt male broiler breeders were improved by using vitamin E-selenium injection in present experiment. Effects of vitamin E and selenium on reproductive traits of male birds were studied previously [15–17]. It is reported that vitamin E combined with selenium provided the best protection against lipid peroxidation in chicken semen compared with vitamin E or selenium standing alone in a diet [18]. Selenium-dependent glutathione peroxidase is an essential component of the antioxidant system in avian semen [9]. In this regard, Flohe L [19] suggested that the main problem to arise in selenium deficient spermatozoa is an imprecise architecture of the sperm midpiece. Experiments with broiler breeders showed that semen quality can be achieved by supplementation of selenium and vitamin E in diet [20,21]. According to the results of Long and Kramer [22], lipid peroxidation is a significant factor affecting the fertility of stored turkey sperm. Besides, they stated that addition of vitamin E alone was not sufficient to deter lipid peroxidation during storage of turkey semen. According to this literature review, it is suggested that vitamin E combined with selenium had an important role to increase the fertility traits in post-molt male broiler breeders in present experiment.

Few studies have been conducted on the use of GnRH analogues in male broiler breeders. However, we found several studies that demonstrate the mechanism action of GnRH analogues in mammals and birds [12,23,24]. GnRH analogues stimulate GnRH receptors in the anterior pituitary gonadotropes, causing FSH and LH production and release [25]. Our results are in accordance with findings of Elnagar SA [26] who reported that the synthetic GnRH (receptal solution) was capable of improving 40 week old cockerel's reproductive status. It is suggested that the endocrine response to GnRH agonist treatment can be characterized by two phases, including acute and chronic phases, which during at acute phase there is an initial hyperstimulation of LH and FSH, followed by an increase in testosterone [23]. Previous studies have shown a reduction in the amplitude of release and concentration of hypothalamic GnRH following a reduction in the synthesis of pituitary FSH and LH in aging male Japanese quails [25,27]. These results are in agreement with Avital-Cohen N [28] who reported that low fertility was accompanied by a reduction in hypothalamic GnRH expression in white leghorn roosters. Ottinger MA [29] described the Neuroendocrine regulation of GnRH and behavior during aging in quails. They noted that qualitative and quantitative alterations in aromatase enzyme system following the change in estradiol secretion regulate the sexual behavior or the control the secretion of GnRH in male quails. Furthermore, aging roosters had lower semen-quality variables, plasma testosterone concentration and mRNA expression of hypothalamic GnRH than young roosters [30]. Therefore, it concluded that the use of GnRH analogue increased the reproductive activity of post-molt male broiler breeders in our experiment.

The serum glucose concentration increased in male broiler breeders injected with GnRHa in the present research. Weil S [31] indicated that plasma insulin levels were higher in low-fertility, aging rooster than in high-fertility roosters. Reduced glucose uptake, in part, appears to be responsible for reduced metabolism and poor motility of sperm in roosters [32]. This study supported the results of Jutte NHPM [33], who reported that Insulin as an endocrine regulator, like FSH, is essential in the regulation of glucose transport and lactate production by

sertoli cells. Thus, it can be stated that the increase in blood glucose levels in this experiment could be related to increase male broiler breeder's fertility.

Results of present experiment showed that liver enzymes activity of post-molt male broiler breeders was not affected by both of vitamin E-selenium and GnRHa injection. In contrast, [4] reported that serum AST and ALT decreased significantly in post-molt male broiler breeders fed vitamin E compared with those fed vitamin C, probiotic and proteins. They stated that lower AST and ALT levels are the indicator of better liver health in animals. A significant reduction in AST and ALT enzymes activities was observed in broiler chicks fed 0.3 ppm selenium [34]. The overall knowledge about the effect of GnRH analogues on the liver enzymes activities in male broiler breeders is limited. Therefore, direct comparisons cannot be made.

The levels of T4 and testosterone were increased in post-molt male broiler breeders injected with either vitamin E-selenium or GnRHa in present research. Khan RU [4] indicated that the serum thyroid hormone concentration increased in post-molt male broiler breeders fed diets supplemented with vitamin E. Thyroid hormones are involved in controlling metabolic rate, and the concentration of circulating T3 is positively correlated with oxygen consumption in broilers [35].The effects of thyroid hormones alterations on the reproductive system have been studied in animals and have generally shown that changes from normal thyroid function resulted in decreased sexual activity and fertility [36]. Direct effect of T4 resulted in minimal oxygen consumption changes in testes following increased amount of testosterone biosynthesis [37]. The underlying reason for increased of T4 in response to GnRH analogues administration in male broiler breeders is not known.

The serum testosterone hormone was increased significantly in post-molt male broiler breeders treated with vitamin E-selenium combined with GnRHa. The positive effect of vitamin E-selenium on testosterone secretion may be associated with testes better utilization of selenium and vitamin E [15]. According to these authors, the increased levels of these antioxidants contributed to the maintenance of the seminiferous tubules and testosterone biosynthesis that is consistent with observations of Golzar-Adabi SH [38] who reported that the plasma testosterone concentration did not alter by using vitamin E supplementation in Japanese quails diet.

In conclusions, the results of this experiment showed that fertility characteristics were improved in post-molt male broiler breeders treated by both vitamin E-selenium and GnRHa. In addition, the levels of serum T4 and testosterone were increased in male birds received vitamin E-selenium and GnRHa. However, more research is needed to clear the mechanism action of GnRH analogues on liver enzymes activities and thyroid hormones in domestic birds.

Conflict of inertest statement

We declare that we have no conflict of interest.

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