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Supplementation of dietary vitamins, protein and probiotics on semen traits and immunohistochemical study of pituitary hormones in zinc-induced molted broiler breeders

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ABSTRACT

The purpose of this study was to investigate the effect of dietary vitamin E and vitamin C, probiotics mixture and protein level and their combination on semen quality and immunohistochemical study of some pituitary hormones in male broiler breeders. One hundred and eighty male broiler breeders 65 weeks old were divided into six groups by completely randomized design. The birds were subjected to zinc-induced molt by mixing zinc oxide at the rate of 3000 mg/kg in the feed. After molting, one group was fed control diet (CP16%). The other groups were fed vitamin E (100 IU/kg), vitamin C (500 IU/kg), probiotics (50 mg/L of drinking water), protein (CP14%) and combination of these components. These treatments were given for five weeks. After the feeding period, semen samples were taken and analyzed for semen volume, sperm concentration, motility and dead sperm percentage. Pituitary samples were collected from three birds per replicate and were processed for immunohistochemical study. The results of semen quality parameters revealed that semen volume and sperm motility were significantly high in the vitamin E fed group, while the dead sperm percentage decreased significantly in the vitamin C group. The morphometric analysis revealed that compared to other groups, vitamin E caused a significant increase in the size and area of FSH, LH gonadotropes and lactotropes. These results showed that vitamin E alone may play some role in the enhancement of semen quality and growth of gonadotropes and lactotropes.

Introduction

Molting is a complex process which is associated with renewal of reproductive organs. It has been previously reported that semen quality and quantity in molted males is influenced by the process of molting (Woodard et al., 1979; Jacquet et al., 1993). Also during the process of molting, significant change in the metabolic and reproductive hormones have been reported (Jacquet et al., 1993; Khan et al., 2011). These findings suggest that the pituitary gland functions are very important in the control of many endocrine functions, which are altered during the molting phase. 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 (Khan et al., 2012a).

Dietary vitamin E and vitamin C are important antioxidants and play a vital role in the improvement of semen quality in male birds (Khan, 2011; Khan et al., 2012b,c). Several reports have been published on the beneficial effects of vitamin E and C on improving semen quality in male poultry (Pardue and Thaxton, 1986; McDaniel et al., 1998; Elansary et al., 1999; Lin et al., 2005; Ceroloni et al., 2006; Biswas et al., 2009). Probiotics are mixed bacterial cultures, which beneficially affect the host by improving the intestinal microbial population (Fuller, 1989; Ayed and Ghaoui, 2011; Houndonougbo et al., 2011; Dibaji et al., 2012). Previously, we found that probiotics in drinking water have some beneficial effects on semen quality in molted broiler breeders (Khan et al., 2012a). Similarly, there are several reports which have documented that low dietary protein improved the semen quality of broiler breeders (Zhang et al., 1999; Romero-Sanchez et al., 2007).

Owing to the key role of the pituitary gland in growth and reproduction of avian species, a research project was designed to determine the effects of vitamins, probiotics, protein levels or the combination of these components on the relationship between the semen traits and an immunohistochemical study of gonadotropes and lactotropes of male broiler breeders after zinc-induced molting.

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Materials and methods

Reagents

Probiotics was obtained as Protexin® from HiltonPharma (the Netherlands). The concentration of the bacteria was 1×10^9 CFU. Vitamin E and C were purchased from BA Traders® (Lahore, Pakistan). The primary antibodies (rabbit raised) used in this study for localization of FSH and LH gonadotropes and latotropes of the adenohypophysis were purchased from the National Hormone and Peptide Program (Harbor UCLA Medical Centre, Torrance, CA, USA). For secondary antibody, Rabbit-specific HRP/DAB detection IHC kit (Abcam, Cambridge, UK) was used. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were used at reagent grade purity.

Figs. 1–3.

Experimental design and diets

All procedures followed in this experiment were strictly adhered to the protocols approved by the University of Agriculture, Faisalabad. This research work involved 180 male broiler breeders aged 65 weeks. The birds were procured from the local breeder farm and delivered to Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. Upon arrival, the birds were weighed and randomly assigned to six groups (five replicates) in equal number by completely randomized design in a floor pen (3.96 m × 3.96 m). Lighting and temperature of the house were automatically controlled. Average temperature of day and night was 25 ± 0.2 °C. The birds were acclimatized for one week, during which they were fed normal breeder ration (CP-16%, 140 g/bird/day) as described by Khan et al. (2012a) and water was available *ad libitum*. Lighting schedule was maintained at 16 h per day and temperature was 25 °C. At the beginning of the second week, birds were molted with zinc oxide (ZnO) at the rate of 3000 mg/kg of feed with moderate decrease in lighting schedule from 16 h to 12 h, and 35 g/bird offered feed on a daily basis (Khan et al., 2012a). The molting phase continued for two weeks during which there was an average reduction of 20% in body weight. After molting, one group was kept as control (CP-16%), the second group was fed vitamin E (100 IU/kg), the third group vitamin C (500 IU/kg), the fourth group probiotics (50 mg/L) in drinking water, the fifth group lower than normal protein level (CP-14%) and the last group was fed a combination of vitamin E, C, protein and probiotics (CP: 14% + 100 IU/kg vitamin E + 500 IU/kg vitamin C + 50 mg/L probiotics). The feeding schedule was continued for 5 weeks and first sampling was conducted in the beginning of week 6 continuing for up to 10 weeks.

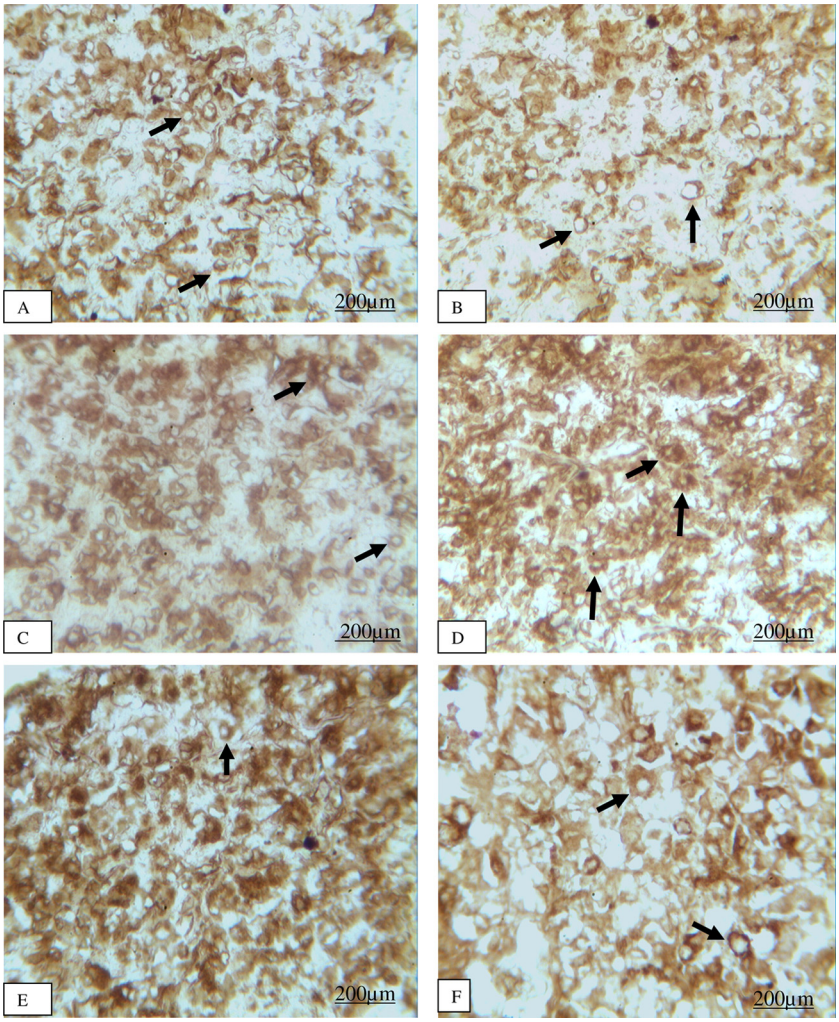

Fig. 1. Immunohistochemical localization of immunoreactive FSH cells of control and treated groups after molting. (A) Control; (B) vitamin E; (C) vtamin C; (D) protein; (E) probiotics; (F) combination. Arrow showing round to elliptical cells with clear nucleus {control and vitamin E, darkly or less darkly stained cells {vitamin C, protein, probiotics and combination. Scale bar = 200 µm.