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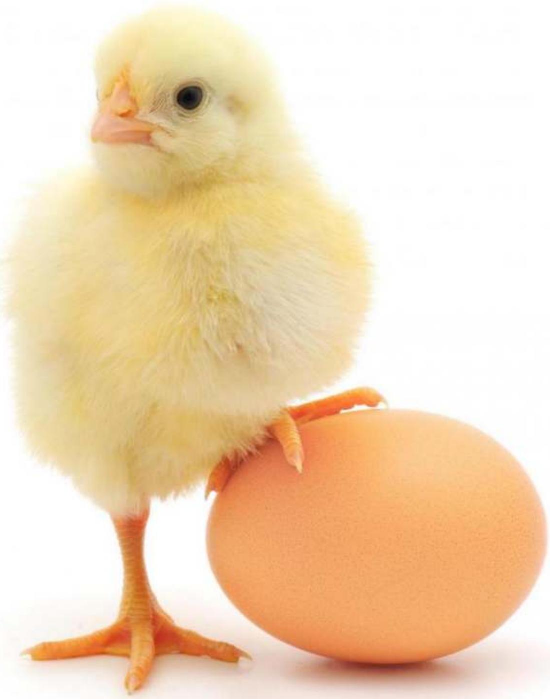

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L-Dopa Feeding Induces Body Growth and Reproductive Conditions in Japanese Quail, *Coturnix coturnix Japonica*

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Abstract: It is obvious from several studies that the different neurotransmitters are associated with various metabolic activities of the body directly or indirectly via their effects on hypothalamic/pituitary hormone but regarding the control of reproductive system catecholamines seem to have a central role. In view of above fact the present study was designed to test whether neurotransmitter precursor L-Dihydroxyphenylalanine (i.e. L-DOPA, a dopamine precursor) feeding, can influence body growth and reproductive conditions in high food value poultry bird Japanese quail, *Coturnix coturnix japonica*. Experiments were performed in both male and female Japanese quail. Sexually immature (one week old) quail chicks were divided into two groups of 24 birds each. Group-I provided with normal diet and served as control while Group-II (experimental group) provided with food mixed with L-DOPA (50 mg/15 gm food). At the age of 3 weeks (when birds were sexually distinguished) Group-I was divided into two sub groups IA (male) and IIA (female) of six birds each. Similarly Group II was sub divided into IB (male) and IIB (female) of 6 birds each. Observations were made up to the age of 5 weeks. Male experimental group (IB) showed significantly increased testicular activity, cloacal gland volume, body weight, plasma testosterone and LH level in comparison to control (IA). Similarly female experimental group (IIB) showed significantly high weight of reproductive organs (uterus, ovary, oviduct, ovarian follicle), body weight, egg weight and size and number of follicles. On the other hand plasma prolactin level was significantly low in comparison to control (IIA). Results suggest that the development and reproduction in Japanese quail might be associated with dopaminergic system of the brain.

Key words: L-DOPA, reproduction, development, japanese quail, poultry bird

Introduction

Neuroendocrine system is an integral component of the neuronal apparatus of the brain, a major portion of which is concentrated in the hypothalamus. According to our present knowledge neuropeptides, a biogenic amines, are the most important agents synthesized and secreted by neuroendocrine cells. The role of Central Nervous System (CNS) neurotransmitters, which appear to be the mediator of many control mechanisms in mammal (Weiner and Ganong, 1978), is little understood in other vertebrate groups except mammals. The hypothalamic monoamines dopamine (3, 4-dihydroxyphenylalanine), noradrenalin and serotonin have profound effects on the release of mammalian pituitary hormone. Presumably in most cases by modulating release of the hypothalamic hormone (Meites *et al*., 1977). The hypothalamus is of paramount importance as an “integration centre” for the various influences on pituitary function. The hypothalamus apparently responds to the exteroceptive and interoceptive stimuli by altering secretion of certain neurotransmitters and release hormones. The response of any given pituitary hormone is presumably the sum of all the stimulatory or inhibitory influences. Ideally a study of the control of pituitary function should not only show the effects of any particular influence on hormone secretion, but should also demonstrate the changes in metabolism of neurotransmitters and release of inhibiting hormones.

Dopamine (DA) is reported to be synthesized largely in CNS. At the time of its discovered in brain, dopamine was proposed to act only as an intermediate in the biosynthesis of norepinephrine and epinephrine. However, in 1958 DA was demonstrated to be present in brain in concentration similar to that of norepinephrine suggesting that dopamine not only serves as a precursor but might function as a neurotransmitter in its own right. When it was discovered that striatum contained 70-80% of the brain dopamine and that depletion of striatal dopamine was important in the pathogenesis of Parkinson’s disease it rapidly became a subject of the intense interest among neuropharmacologists. Injections of dopamine (DA) into the third ventricle also result in an increase of the peripheral plasma testosterone. This rise in testosterone level was also noted after intraventricular DOPA administration. So the effect of exogenous DA was similar to that of the endogenous amine synthesized in brain after the injection of its precursor, possibly reaching to brain via blood-brain barrier.

A prominent neurotransmitter affecting prolactin (PRL) secretion is dopamine (DA). DA inhibits PRL release from chicken, pigeon and turkey anterior pituitary cells *in vitro* (Fehrer, 1984; Hall and Chadwick, 1983; 1984; Harvey *et al*., 1982) and in turkeys in vivo (El Halawani *et al*., 1991). PRL release prompted by electrical stimulation of the turkey brain is curtailed by the DA agonist apomorphine and potentiated by the DA antagonist pimozide (El Halawani *et al.*, 1991). Intraventricular administration of DA increases PRL level in nonlaying turkey hens, while it has no effect in incubating ones (Hargis and Burke, 1986). DA-binding sites in the anterior pituitary of bantam hens decrease during incubation, when PRL is high (Macnamee *et al.*, 1986). The dopaminergic D_2 receptor agonist quinpirole inhibits VIP-induced PRL secretion and PRL mRNA level when incubated with turkey anterior pituitary cells (Xu *et al.*, 1996), suggesting the presence of inhibitory D_2 DA receptors within the pituitary. DA Stimulates VIP release from isolated, purified turkey hypothalami in a dose-dependent manner and this VIP release is completely suppressed when the D_1 DA receptors are blocked (Chaiseha *et al.*, 1997). Central infusion of DA into the turkey brain at low concentration results in significant increase in plasma PRL (Youngren *et al.*, 1995; 1996; 1998a). DA affects this PRL increase by way of D_1DA receptors residing within the avian hypothalamus (Chaiseha *et al.*, 1997; Youngren *et al.*, 1996). When an identical concentration of DA is infused into the pituitary of the turkey, no rise in plasma PRL is observed; however, the PRL increases in response to electrical stimulation of the medial preoptic area (ES/POM) is suppressed by the DA infusion. ES/POM and central infusion of DA appear to increase plasma PRL by releasing VIP from the hypothalamus. In turkeys immunized against VIP, neither ES/POM nor central DA infusion will increase plasma PRL (Youngren *et al.*, 1994; 1996). The infusion of VIP into the turkey pituitary effects a rapid and substantial increase in plasma PRL (Youngren *et al.*, 1998b), an increase that is completely suppressed when DA is infused in conjunction with VIP (Youngren *et al.*, 1998a). DA appear to block the VIP-stimulated release of PRL by activating inhibitory D_2 DA receptors in the anterior pituitary.

Egg size represents a key, hormonally mediated, life-history trait which varies markedly among species (from 0.25 g in bee hummingbirds, *Mellisuga helenae,* to 1500 g in the ostrich, *struthio camelus*). Most of the variation in egg size within species is due to difference in the mean egg size produced by individual females, i.e. it is independent of variation in environmental, nutritional and social conditions (Reed, 2000). Experimental manipulation of hormone levels can be a powerful tool not only to understand the physiological basis of phenotypic variation in hormonally-mediated traits, but also the ecological and evolutionary consequences of this variation (Ketterson and Nolan, 1999; Sinervo and Basolo, 1996). Hormones of the Hypothalamo-Pituitary-Gonadal (HPG) axis regulate ovarian function in birds and other oviparous vertebrates. Our knowledge of this system (Norris, 1997; Williams, 1999) allows for the identification of several 'candidate' hormones that might be involved in regulation of egg-size variation; principally the gonadotropins, Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) and the steroid hormones (estradiol and progesterone). It is clear that certain hormonal manipulations (in particular antiestrogens and FSH) can modify expression of the egg size phenotype while maintaining the range of inter-individual variation in this trait. Several studies have shown that manipulation of egg size results in a corresponding, often compensatory, adjustment in clutch size.

In view of our interest in the neurotransmitter induced developmental and reproductive changes of avian species present study was investigated in a high food value poultry bird, Japanese quail, *Coturnix coturnix japonica*. Experiments were preformed in both male and female Quails following L-DOPA (L-dihydroxyphenylalanine, a precursor of dopamine) feeding.

Materials and Methods

The Japanese quail belongs to the order Galiformes, genus *coturnix* and species *japonica*. The scientific designation for Japanese quail is *coturnix japonica*. Adult females have pale breast feathers that are spackled with dark coloured spots. Adult males have uniform dark rust red feathers on the breast and cheek. Sex differences in plumage colour appears about 3 weeks of age. The Japanese quail was used first as a singing bird and then as an egg producer. It later became popular as a laboratory animal, because of its small body size, little consumption and rapid maturation. Hatching eggs were collected from the breeding cages of Japanese quail maintained in bird room of animal house of our department and loaded them in the incubator. Suitable incubation environment were selected to hatch quail eggs successfully. Dry bulb thermometer was set at 37.5°C and wet bulb at 29.3°C and 32.2°C in the incubation and hatching compartments respectively. Chicks were hatched successfully on 18th day. Special chick ration, cool and clean water were provided at all times.

Experiments: One week old (sexually immature) quail chicks were divided into two groups of 24 birds each. Group-I provided with normal diet and served as control while Group-II (experimental group) provided with food mixed with L-dihydroxyphenylalanine* (L-DOPA, a dopamine precursor, 50 mg/15 gm food). At the age of 3 weeks (when birds were sexually distinguished). Group-I was divided into two sub groups IA (male) and IIA (female) of six birds each. Similarly Group II was sub divided into IB (male) and IIB (female) of 6 birds each. Observations were made up to the age of 5 weeks. For the purpose of observations egg laying size and weight of control groups IA and IIA were kept together by marking them with a particular identification (numbering tag in the leg). Similarly experimental group IB and IIB were also kept together.

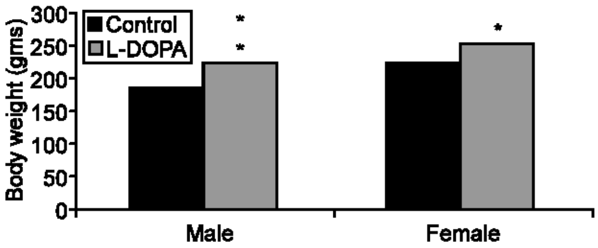

Fig. 1: Effect of L-DOPA feeding on body weight of male and female Japanese quail. Values are Mean±SE. Significance of difference from control (*p<0.05, **p<0.001; Student's 't' test)

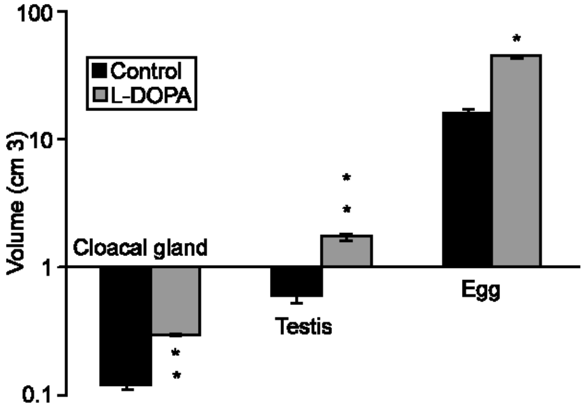

Fig. 2: Effect of L-DOPA feeding on cloacal gland, egg and testicular volume of Japanese quail. Values are Mean±SE. Significance of difference from control (*p<0.05, **p<0.001; Student's 't' test)

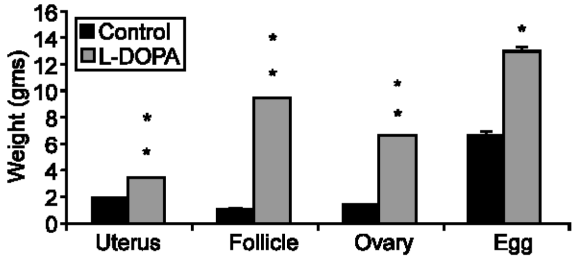

Fig. 3: Effect of L-DOPA feeding on weight of eggs, follicles, ovary and uterus of female Japanese quail. Values are Mean±SE. Significance of difference from control (*p<0.01, **p<0.001; Student's't' test)

Male: After 5 weeks of L-DOPA feeding birds (male, control-IA and experimental-IB) were anesthetized with ether. Blood was collected from left Ventricle of heart and RIA (Chastel *et al.*, 2005) was performed for testrosterone and LH. Volume of cloacal gland and testes were calculated by measuring long and short axis (with the help of scale and divider) and by using Bissonet formula $4/3\ \pi ab^2$ (where a=½ of the long axis b = ½ of the short axis). Growth of body was assessed by body weight (in gm). Testes were removed and processed for histological studies.

Female: Regular observations were made on the egg laying by female birds (Control-IIA and experimental-IIB). Egg layed were weighed and volume was also taken with the help of Bissonet formula. After 5 week of L-DOPA feeding bird of each group were weighed and anesthetized with ether. Blood was collected from left Ventricle and RIA (Boswell *et al.*, 1995) was performed for prolactin. Reproductive organs (uterus, ovary, oviduct and ovarian follicles) were removed by dissecting birds and weighed. Follicular sizes were measured with the help of divider and scale. Numbers of follicles were counted.

Animal housing, care and application of experimental procedures were in accordance with institutional animal ethic guidelines. Values from all the above groups were expressed in terms of mean±S.E. statistical analysis was done with the help of student's 't' test (Bruning and Kintz, 1977).

*L-DOPA: M.W. 197.19, Batch No. J01Z/ 1592/ 2807/ 62/ Product No. 47079, S. D. Fine Chemicals Ltd.

Results and Discussion

Male: Cloacal gland (an androgen dependent sex accessory), testicular volume (Fig. 2), plasma testosterone and LH level (Fig. 4) and Body weight (Fig. 1) of L-DOPA fed group showed significantly (p<0.001) higher values when compared to the control. The size of cloacal gland correlated with plasma LH and testosterone level and testicular size.

Histologically, the testis of control bird, sacrificed at the 5th week showed moderate activity while the testis of L-DOPA fed bird showed full breeding condition. Enlarged seminiferous tubules had some stages of spermatogenesis with spermatozoa and interstitial spaces contained active leyding cells (Plate 1).

Female: Maturation of female reproductive organs (uterus, follicles and ovary) of L-DOPA fed group showed significantly (p<0.001) higher values when compared to the control (Fig. 3). L-DOPA fed group started egg laying at 3rd week. While in control group first egg laying was noticed at 5th week. Eggs layed at the 5th week were collected from both the groups (female, control-IIA and experimental-IIB) and comparison were made. The size and weight of the eggs layed by L-DOPA fed quail were significantly higher (p<0.001) than those of control group (Fig. 2 and 3, Plate 2). The follicular size and number are also significantly higher (p<0.01) than that of control (Fig. 5, Plate 3). Similarly the body weight of experimental group show significantly higher values (p<0.05) when compared to the control (Fig. 1).

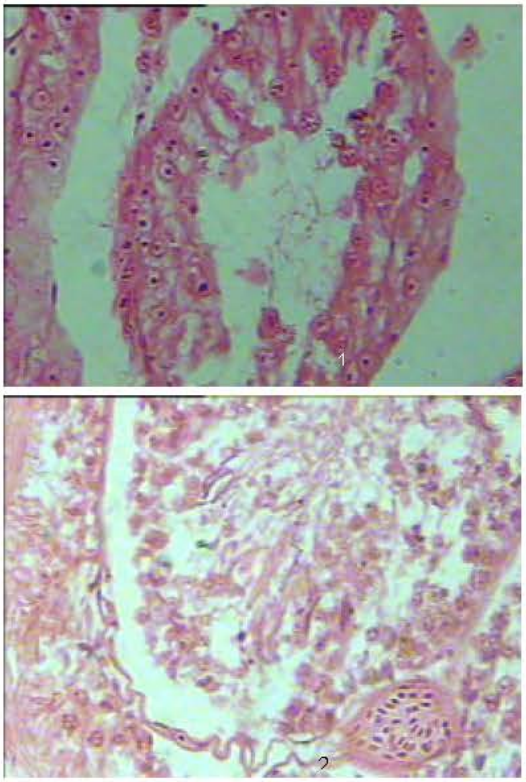

Plate 1: (I) Photomicrograph showing the moderate condition of T.S. of testis of control group 40X, (ii) Photomicrograph of T.S. of testis of L-DOPA fed group showing hyperactivity and full breeding conditions with stages of spermatogenesis. Spermatozoa and active Leydig cells are clearly seen. 40X

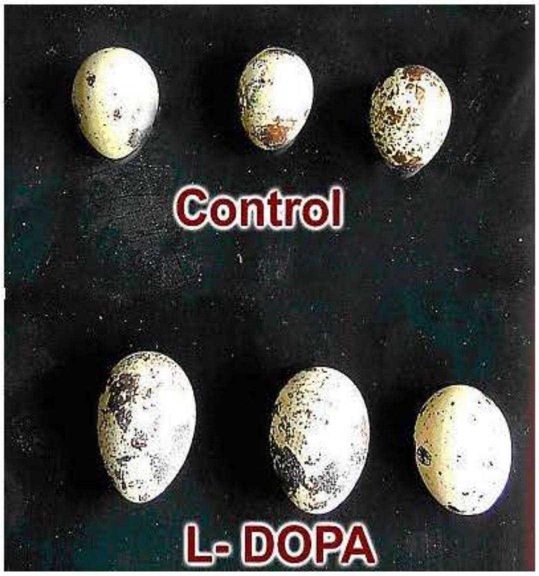

Plate 2: Photograph showing significant ($p<0.01$) difference of the eggs size between control and L-DOPA fed group

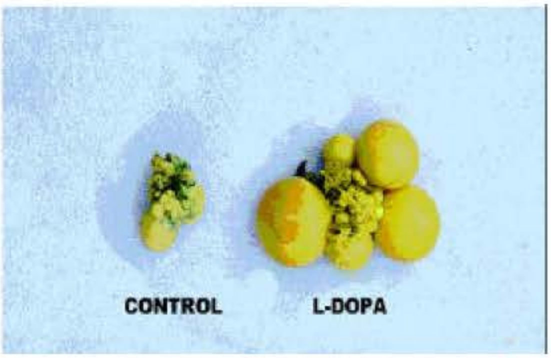

Plate 3: Photograph showing significant ($p<0.01$) difference of follicular size and number between control and L-DOPA fed group

In contrast to body weight and reproductive organs in female, plasma prolactin level was significantly ($p<0.001$) lowered in L-DOPA fed group (Fig. 4).

Brain monoamines are reported to regulate up to some extent the activity of hypothalamic factor which in turn regulate the pituitary gonadotropins (Lu *et al.*, 1977). In general, hypothalamic norepinephrine (NE) is known to stimulate whereas serotonin is found to inhibit the release of gonadotropin (Meites *et al.*, 1977). NE has been reported to elicit the release of hypothalamic decapeptide, gonadotropin releasing hormone (GnRH), which in turn causes the reduction in hypothalamic catecholamine (CA) content and an increase in serotonin turnover occur with the progress of age in male (Simpkins *et al.*, 1977) and female rats (Huang and Meites, 1975) showing gradually decreasing reproductive activities. High serotonin concentration in certain areas of brain (hypothalamus, midbrain and medulla) has also been reported in sexually regressed scotosensitive hamsters. This seasonal reproductive condition may be induced by tryptotophan feeding in photorefractory hamsters and is associated with high serotonin concentration and turnover rate (Wilson and Meier, 1983). Retardation in reproductive activity in aging rat is also correlated with hypothalamic deficiency of catecholamine and can be corrected with administering L-DOPA, epinephrine or improniazid (Quadri *et al.*, 1973). Maintenance of estrous cycle in aging female rat is also reported to be influenced by centrally acting drug like L-DOPA which alters catecholamine level (Forman *et al.*, 1980) and scotosensitivity in Syrian hamster by tryptophan feeding (Wilson and Meier, 1983).

It has been reported that elevating the level of the amino acid L-Dihydroxyphenylalanine (L-DOPA), in the blood stream of poultry (by incorporation in the food) has the effect of reducing the cholesterol content in the eggs

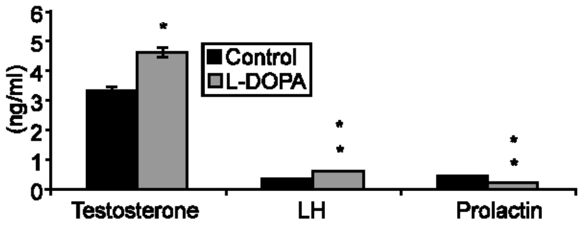

Fig. 4: Effect of L-DOPA feeding on plasma testosterone, LH and prolactin level of Japanese quail. Values are Mean±SE. Significance of difference from control (*p<0.01, **p<0.001; Student's 't' test)

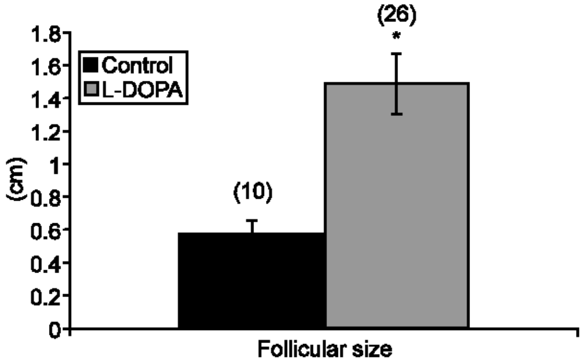

Fig. 5: Effect of L-DOPA feeding on size and number of follicles in female Japanese quail. Values are Mean±SE. Significance of difference from control (*p<0.01, Student's 't' test). The number in the brackets present on the bar indicates the total number of follicles in each group

produced by such poultry and also has the effect of reducing the ratio of saturated to unsaturated fatty acids in the eggs produced by such poultry.

It has also been reported that the injections of DA into the third ventricle of rat reduced serum prolactin values and increased PIF activity in the portal circulation. A single systematic injection of DA failed to alter serum PRL concentration in rat (Shaar and Clemens, 1974). But, it was also shown that infusion of relatively large doses of DA via a carotid arotic cannula depressed prolactin (Shaar and Clemens, 1974). Systemic injection of L-DOPA, the precursor of DA, was found to inhibit prolactin release in rat (Shaar and Clemens, 1974). There is evidence that L-DOPA acts to depress this release only after being converted to DA (Jimenz *et al.*, 1978). DA agonists (apomorphine) have also been shown to inhibit PRL release (Mueller *et al.*, 1976). Administration of drugs that depress hypothalamic DA activity result in an increase of PRL secretion. Injection of α-methyl-p-tyrosine, α-methyl-m-tyrosine or methyldopa all inhibitors of catecholamine synthesis, promote elevation of serum PRL (Koch *et al.*, 1970). The agonists of DA are also been reported to inhibit TSH and stimulate GH release. Pulsatile GH secretion is still evident with an increased number of secretory bursts in rat with a complete hypothalamic island produced by sectioning with Halasz knife. This technique depletes hypothalamic norepinephrine content without affecting dopamine content (see review Weiner and Ganong, 1978).

Other studies in the field of avian reproduction have made an interesting suggestion that the seasonality of reproduction in many more avian species may be determined by specific temporal relation between serotonin and dopamine neurotransmitter precursors (5-HTP and L-DOPA). Testicular and ovarian development and fat deposition are stimulated or inhibited after daily injections of 5-HTP and L-DOPA at the interval of 12 or 8 hours respectively, in the white throated sparrow *Zonotrichia albicollis* (Miller and Meier, 1983), Japanese quail *Coturnix coturnix japonica* (Chaturvedi and Meier, 1988), the migratory red headed bunting, *Emberiza bruniceps* (Chaturvedi and Bhatt, 1990), non-migratory spotted munia, *Lonchura punctulata* (Chaturvedi and Prasad, 1991; Prasad *et al.*, 1992), Lal munia *Estrilda amandava* (Chaturvedi *et al.*, 1994) and Indian weaver bird *Ploceus philippinus* (Prasad and Chaturvedi, 2006). Injections of 5-HTP and L-DOPA given at different intervals showed marked changes in certain blood and serum constituents (Prasad and Thakur, 2005; Prasad *et al.*, 2006) hormonal level (Prasad and Roy, 2003; Prasad *et al.*, 2003) and neurosecretory materials of the median eminence (Prasad and Rathore, 2004).

The effect of L-DOPA feeding in Japanese quail is evident from the results of the present experiments. Earlier studies also revealed that the dopaminergic activity, as one of the circadian oscillators, may influence neuroendocrine gonadal axis (Bhatt and Chaturvedi, 1993). It is suggested that high level of dopamine resulted from DOPA feeding possibly helped to induce and maintain reproductive conditions. DOPA feeding is reported to cause significant increase in brain dopamines (Wilson and Meier, 1989). The brain monoamines have been reported to modulate pituitary gonadotrophin release (Meites *et al.*, 1977). From the finding of the present study it may be suggested that L-DOPA feeding induced the synthesis of dopamine in the brain and high dopaminergic activity affected the neuroendocrine-gonadal axis resulting changes in the body growth, reproductive conditions (Cloacal gland, testicular volume, egg laying, egg weight, egg size, uterus, follicles and ovary) and plasma hormonal levels (luteinizing hormone, testosterone and prolactin) in sexually immature, high food value poultry bird, Japanese quail, *Coturnix coturnix japonica*.

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