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RESEARCH **P**APER

Effect of serum leptin concentration on cognitive ability of male and female Vanaraja chickens

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In males, the intelligent birds had significantly higher level of serum leptin than the dull birds (0.62 ± 0.06 vs. 0.42b ± 0.08ng/ml). Similar trend was also visible in female birds (1.03 ± 0.13 vs. 0.78b ± 0.12 ng/ml). Leptin acts to facilitate long-term potentiation in the hippocampus, a process important for memory processing, which was evident from the present study. Hence, this may be a reliable indicator of cognition. Females had a higher level of leptin concentration in serum than males, but that did not necessarily reflect their performance in terms of cognitive and conditioning abilities. This might be due to higher adiposity in females than males.

Key words : Serum leptin concentration, Cognitive ability, Vanaraja

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INTRODUCTION

Cognition usually refers to the cognitive mechanisms involved in learning, memory, and decision making. Leptin is the satiety hormone, is a hormone made by adipose cells that helps to regulate energy balance by inhibiting hunger. Leptin is opposed by the actions of the hormone ghrelin, the "hunger hormone". Both hormones act on receptors in the arcuate nucleus of the hypothalamus to regulate appetite to achieve energy homeostasis. In obesity, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores. The blood–brain barrier (BBB) regulates the blood-to-brain passage of gastrointestinal hormones, thus, informing the brain about feeding and nutritional status (Farr *et al*., 2006). Disruption of this communication results in dysregulation of feeding and body weight control. Leptin, which crosses the BBB to inform the CNS about adiposity, provides an example. Impaired leptin transport, especially coupled with central resistance, results in obesity. Adiposity and cognition, controlling for the potential effects of age, gender, and possession of the APOE ε 4 allele. Specific hypotheses included: (i) higher BMD would be significantly associated with better current and future cognitive functioning, particularly verbal memory; (ii) higher adiposity and lower lean body mass would be related to current cognitive function and predict subsequent cognitive function.

RESEARCH METHODOLOGY

Experimental design :

The experimental birds were randomly divided in to three groups.

– T_1 F: Female Vanaraja – 50 birds
– T_2 M: Male Vanaraja - 50 birds
– T_3 M+F: (Male + Female) Vanaraja- 25 each = 50 birds.

Leptin assay :

The serum leptin concentration was assayed by LSBio Sandwich enzyme immunoassay Kit (LifeSpan Biosciences™, USA), Catalog No. LS-F5794 (Wagner *et al.,* 1996)

Sample preparation :

Serum collection :

Blood was collected by a serum separator tube and sample was allowed to clot for two hours at room temperature. The the samples were centrifuged for 20 minutes at approximately 1000 x g. Then they were stored in cryovials at -20°C for later use.

Preparation of standard :

The Standard was reconstituted with 0.5 ml of Standard Diluent, kept for 10 minutes at room temperature, shaken gently. The concentration of the standard in the stock solution was 2,000 pg/ml. Seven tubes were prepared containing 0.25 ml Standard Diluent and a double dilution was produced in a series to set up 7 points of diluted standard such as 2,000 pg/ml, 1,000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml and the last EP tubes with Standard Diluent is the blank as 0 pg/ml.

Detection Reagent A and Detection Reagent B - The stock Detection A and Detection B were briefly centrifuged before use. The working concentration was diluted with Assay Diluent A and B, respectively (1:100).

Preparation of wash solution :

20 ml of wash solution concentrate (30x) was diluted with 580 ml of deionized or distilled water to prepare 600 ml of wash solution (1x).

Preparation of TMB substrate :

The needed dosage of the solution was aspirated with sterilized tips.

Assay procedure :

– Wells were marked for diluted standard, blank and sample. 7 wells were prepare for standard, 1 well for blank.
– 100 µl each of dilutions of standard (read Reagent Preparation), blank and samples was added into the appropriate wells.
– Plate sealer was covered on the assay plate.
– Plate was incubated for 2 hours at 37 °C.
– The liquid of each well was removed.
– 100 µl of detection reagent a working solution was added to each well.
– Incubation for 1 hour at 37°C was done after covering it with the plate sealer.
– The solution was aspirated and washed with 350µl of 1x wash solution to each well and was allowed to sit for 1-2 minutes.
– The remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper.
– Washing was done for 3 times.
– After the last wash, any remaining wash buffer was removed by aspirating or decanting.
– The plate was inverted and blotted against absorbent paper.
– 100µl detection reagent B working solution was added to each well.
– Then the plate was incubated for 30 minutes at 37°C after covering it with the plate sealer.
– The aspiration process was repeated for total 5 times as done previously.
– 90 µl of substrate solution was added to each well and covered with a new plate sealer.
– Incubation was done for 15 - 25 minutes at 37 °C protecting from light.
– The liquid turned blue by the addition of substrate solution.
– 50 µl of stop solution was added to each well and the liquid turned yellow.
– The liquid was mixed by tapping the side of the plate.
– Any drop of water and fingerprint on the bottom of the plate was removed.
– Observation was taken at 450 nm wavelength immediately.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized

under following heads :

Serum leptin concentration and cognition level :

The serum concentration of leptin in Vanaraja birds is illustrated in Table 1 and Fig. 2. The birds which were cognitively superior or intelligent showed a significantly high ($p<0.05$) level of serum leptin concentration as compared to dull birds in both male and female birds. In males, the intelligent birds had significantly higher level of serum leptin than the dull birds (0.62 ± 0.06 vs. 0.42b ± 0.08ng/ml). Similar trend was also visible in female birds (1.03 ± 0.13 vs. 0.78b ± 0.12 ng/ml).

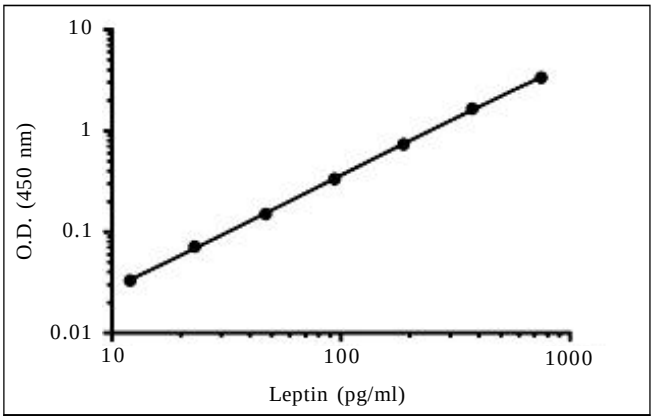

Fig. 1 : Standard curve for chicken leptin assay

Table 1 : Serum leptin concentration in Vanaraja birds on the basis of level of cognition

	Intelligent	Dull
Male (ng/ml)	0.62^{aA} ± 0.06	0.42^{bA} ± 0.08
Female (ng/ml)	1.03^{aB} ± 0.13	0.78^{bB} ± 0.12

Values bearing different superscripts in small letters within a row and capital letters within a column differed significantly ($P<0.05$)

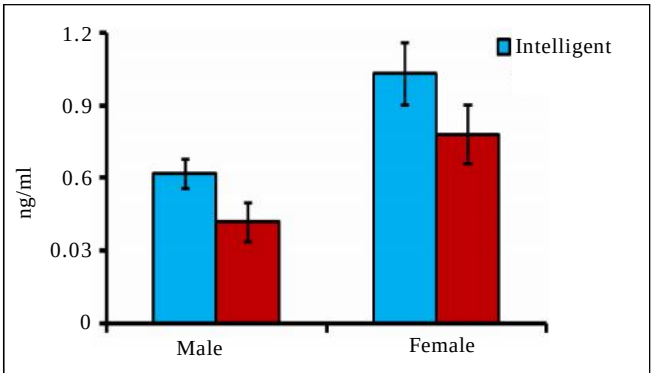

Fig. 2 : Serum leptin concentration in male and female birds on the basis of level of cognition

Female birds had a significantly higher level ($p<0.05$) of leptin as compared to male birds in both irrespective of the performance of the birds.

Leptin level and cognition :

The birds which were cognitively superior or intelligent showed a significantly high ($p<0.05$) level of serum leptin concentration as compared to dull birds in both male and female birds. Female birds had a significantly higher level ($p<0.05$) of leptin as compared to male birds in both irrespective of the performance of the birds.

The present findings lied in the range of serum leptin level as reported by Ngernsoungnerna *et al*. (2012), who found the concentration 0.69 ± 0.15 ng/ml in nonlaying native Thai chicken. The high level of serum leptin in cognitively superior birds corroborated the reports of Gouras *et al*. (2000) who opined that leptin receptors exist throughout the brain including the hippocampus, an area of the brain involved in learning and memory. This can further be confirmed form the observations of Cassy *et al.* (2004) and Raver *et al.* (1998) who stated that leptin acts to facilitate long-term potentiation in the hippocampus,a process important for memory processing. Farr *et al*. (2006) while working with SAM-P8 male mice reported that leptin improves memory processing in the hippocampus. When given immediately, but not 24 h after training, leptin was able to improve retention in both T-maze footshock avoidance and step down inhibitory avoidance.

The low level of leptin in Vencobb birds as compared to Vanaraja might be due to the fact that the former is a commercial broiler, whereas Vanaraja is reared as backyard bird. The present finding well supported the reports of Taouis *et al*. (2001) who stated broiler birsd's rapid growth rate led to excessive body fat associated with impairment of total body metabolism and hypoleptinemia. The mechanisms regulating food intake differ between Vencobb and Vanaraja, presumably because of differential genetic selection for growth rate.

Outside the hypothalamus, where leptin plays a key role in energy expenditure and food intake, leptin improves memory processing in the hippocampus. Leptin plays a role in memory and suggests leptin may contribute to memory impairment in diseases where leptin deficiencies or resistances occur (Farr *et al.*, 2006).

Systemic leptin concentrations increase during puberty in human males and females but the increase is

only maintained in females since leptin concentrations decrease after puberty in males (Spicer, 2001). The decrease in leptin levels after puberty in males is due to testosterone inhibition of leptin secretion (Horlick *et al.*, 2000).

The relative amount of adipose tissue was much higher in female, regardless of BMI, than it was in male. This mass effect, in combination with an increased rate of leptin secretion per unit mass in female, seems to be the major mechanism responsible for the fact that female, at all levels of body fat, have higher circulating leptin levels than do males (Hellstrom *et al.*, 2000). Serum leptin levels vary more directly with total body fat content than with BMI (Rosenbaum *et al*., 1996). Female birds tended to have more adiposity, reflected a higher leptin level as compared to males.

Conclusion :

In males, the intelligent birds had significantly higher level of serum leptin than the dull birds (0.62 ± 0.06 vs. 0.42b ± 0.08ng/ml). Similar trend was also visible in female birds (1.03 ± 0.13 vs. 0.78b ± 0.12 ng/ml). Female birds had a significantly higher level ($p<0.05$) of leptin as compared to male birds in both irrespective of the performance of the birds.

Leptin acts to facilitate long-term potentiation in the hippocampus, a process important for memory processing, which was evident from the present study. Hence, this may be a reliable indicator of cognition.

Females had a higher level of leptin concentration in serum than males, but that did not necessarily reflect their performance in terms of cognitive and conditioning abilities. This might be due to higher adiposity in females than males.

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