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Effect of dietary tryptophan supplementation on oviductal (magnum) morphology in layer chicken

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Abstract
The present investigation was carried out to study the effect of supplemental tryptophan on oviductal histomorphological structures of white Leghorn layers. A total of 350 White Leghorn layers of 18 weeks were allocated to seven experimental groups, each of which included 5 replicates and reared upto 45 weeks of age. Experimental diets consisted of two protein diets along with 5 supplemental levels of tryptophan. The basal diet consisted of normal protein (CP-17 %) with 0.165 % tryptophan and low protein diet with 0.153 % tryptophan. Tryptophan was supplemented at 0.012, 0.015 and 0.035 % in normal protein diet to obtain 0.165 %, 0.18 % and 0.20 % digestible tryptophan and at 0.027 %, 0.047 % in low protein diets (CP- 16.23 %) to obtain 0.18 % and 0.20 % digestible tryptophan respectively. At the end of the study, six birds per treatment were dissected and morphological parameters such as total weight and length of oviduct were measured. For histological studies, after tissue preparation and staining with H and E, histological layers of magnum were recorded. The results of the present study indicated that the total weight and length of various segments of oviduct did not differ by tryptophan supplementation. In his to metrical studies, the length of primary mucosal folds of magnum was not altered by tryptophan supplementation.

Keywords: Layers, tryptophan, magnum and H&E

Introduction
The avian oviduct is a complex biological organ that undergoes a series of hormonal, neural, biochemical and cellular changes during the formation of an egg. It is of special interest to the commercial egg industry. Any alteration or deviation in the function of the oviduct of a laying hen can directly affect egg and egg shell quality. Decline in egg and egg shell quality costs the egg industry millions of dollars every year. The avian oviduct has been studied extensively in some poultry birds, especially the domestic fowl [1, 2]. The avian oviduct is divided into five regions, namely infundibulum, magnum, isthmus, uterus or tubular shell gland and vagina. The infundibulum forms a strong perivitelline membrane and chalaza around the egg yolk, the magnum is responsible for the synthesis and secretion of albumen, the isthmus forms a fibrous membrane around the egg white, the uterus forms the egg shell and finally the vagina connects the uterus to the cloaca. Histological development of the oviduct was observed maximum at the time of egg laying.
The increase of digestible tryptophan: digestible lysine ratio favoured the number of secondary folds in the uterus of layers resulting in higher production of mucus and albumen in the magnum. This leads to reduction in the egg formation time thus, increasing production, weight, mass, conversion in mass and egg dozens [3].
The aim of our present experiment was to study the anatomy and the histological features of magnum in layer hens due to tryptophan supplementation.

Materials and Methods
Three hundred and fifty Single Comb White Leghorn layers of 16 weeks were procured from commercial breeding farm (Namakkal) and the experiment was conducted from March to October 2016. All the birds were reared under standard management conditions throughout the experimental period. Birds were vaccinated against Ranikhet disease (RDVF1) and Infectious Bronchitis (IB).

The experimental layer diets were formulated according to the breeder's specification (Venkateshwara Hatcheries Private Limited). In commercial formulation, the levels of essential amino acids were fixed in relationship with lysine, however in our experiment the essential amino acids were fixed based on digestible tryptophan.

The experimental groups and their diets are as follows

Treatment	Diets	No of birds	No of replicates	No of birds / replicates
Diet I (T_1)	Layer diet with 17 % CP and 0.165 % digestible tryptophan	50	5	10
Diet II (T_2)	Layer diet with 16.23 % CP and 0.153 % digestible tryptophan	50	5	10
Diet III (T_3)	Diet II+ 0.012 % digestible tryptophan supplementation	50	5	10
Diet IV (T_4)	Diet I+ 0.015 % digestible tryptophan supplementation	50	5	10
Diet V (T_5)	Diet I+ 0.035 % digestible tryptophan supplementation	50	5	10
Diet VI (T_6)	Diet II+ 0.027 % digestible tryptophan supplementation	50	5	10
Diet VII (T_7)	Diet II+ 0.047 % digestible tryptophan supplementation	50	5	10

The ingredients of the diets are presented in the Table 1.

At the end of the experiment (45th week), six birds per treatment were randomly selected and slaughtered. The whole oviduct was quickly dissected out, stretched on tray and was studied for gross morphometry. The gross morphometrical study included the total length and weight of oviduct and the individual length of various segments of oviduct was measured using scale. After tissue preparation and H&E staining [4], histological layers of magnum were recognized and primary folds of tunica mucosa with micrometry method were measured. Photographs of the prepared slides were taken with microscope equipped with a camera (Zeiss primostar, Germany with Axiocam ERC5S camera).

Results

The effect of supplementation of tryptophan on morphometrical parameters of oviduct and primary fold length of magnum are presented in Table 2 and 3.

The gross morphology of oviduct including infundibulum, magnum, isthmus, uterus and vagina of layer chicken showed normal morphometry. The mean length (cm) of infundibulum, magnum, isthmus, uterus, vagina, total oviduct and the mean total oviductal weight (g) among all the birds were in normal range and agree with report by [5] in 12-18 months and [6] in 8-11 months old layer birds. No change in length of infundibulum, magnum, isthmus, uterus and vagina and total oviduct weight.

The histology of magnum of layer chicken showed normal histological structures (Plate 1). The magnum showed tall and broad mucosal folds with secondary folds. The tunica mucosa of the magnum was lined by pseudostratified ciliated columnar epithelium with numerous goblet cells. The mean lengths of primary folds (µm) of magnum in our study were in normal range of 3150.5 ± 3.7 to 3201.8 ± 2.6, and our findings are in agreement to [5] in 12-18 months layers. In our study tryptophan supplementation till 45th week of age in layers had no influence in primary fold length of magnum of oviduct.

Our findings were contrary to [3] who observed increased magnum folds in laying hens supplemented tryptophan at 0.183, 0.199 and 0.215 %.

The histomorphological results indicated that tryptophan supplementation did not cause any histological changes in oviduct morphology in layers.

In conclusion, currently protein sources in layer diets have been partially replaced by industrial amino acids such as tryptophan which leads to increased egg mass and production. But still it could be inferred from the above study that tryptophan supplementation had no effect on magnum primary fold length.

Table 1: Ingredients composition (%) of pre-layer and layer diet fed different levels of tryptophan and crude protein

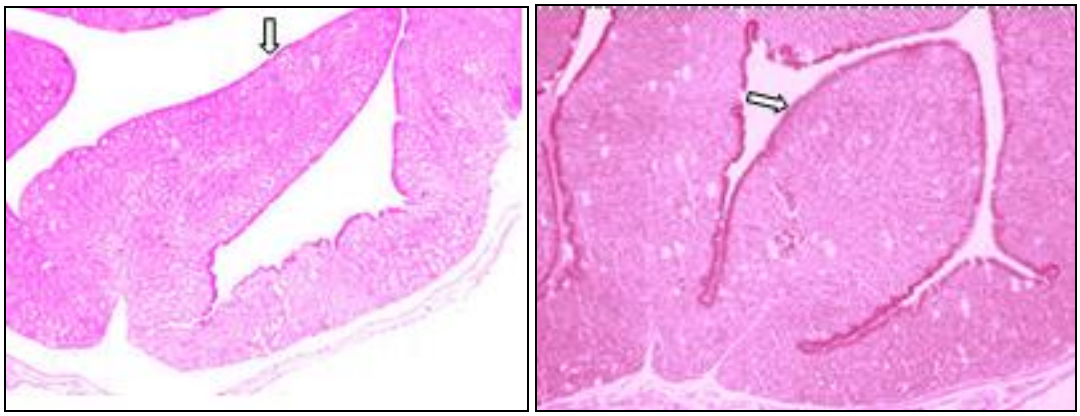
Ingredients (%)	Pre-layer diet		Layer diet	
	Diet I (normal protein)	Diet II (low protein)	Diet I (normal protein)	Diet II (low protein)
Maize	50.8	52.5	49.6	52.2
Deoiled rice bran	15.0	15.0	15.0	15.0
Sunflower oil cake	8.9	10.0	5.1	4.3
Soyabean meal	15.6	12.4	16.5	14.7
Fish meal	3.0	3.0	3.1	3.2
Di calcium phosphate	0.7	0.7	0.56	0.58
Calcite	4.0	4.0	4.0	4.0
Shell grit	1.8	2.0	5.7	5.7
DL-Methionine (g/100kg)	89	96	141	165
Lysine (g/100kg)	8	87	0	34
L-Threonine (g/100kg)	0	0	34	62
Soda Bicarb (g/100kg)	33	27	55	61

Table 2: Mean (± SE) morphometrical parameters of oviduct in White Leghorn layers fed different levels of tryptophan and crude protein at 45th week of age

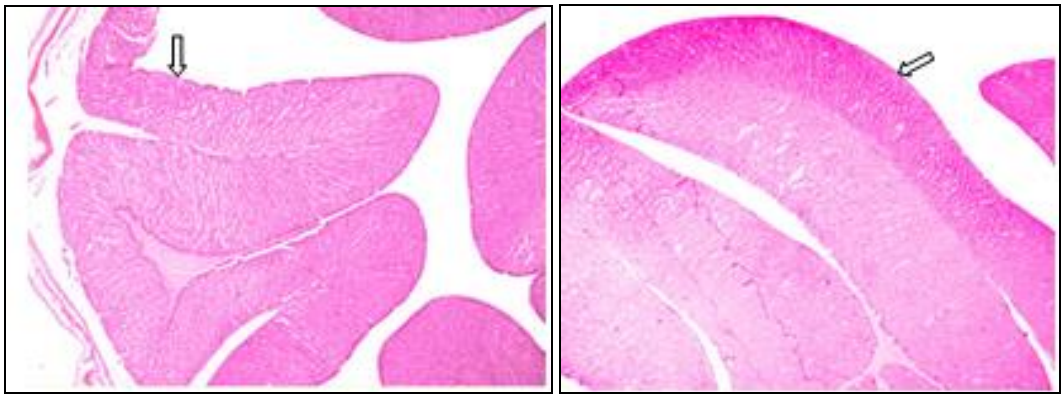
Treatment	Infundibulum length (cm)	Magnum length (cm)	Isthmus length (cm)	Uterus length (cm)	Vagina length (cm)	Total length (cm)	Total weight (g)
T1	11.2 ± 0.6	32.1 ± 0.5	14.5 ± 0.4	7.2 ± 0.2	6.3 ± 0.1	71.3	51.9 ± 0.6
T2	11.0 ± 0.5	32.3 ± 0.4	14.5 ± 0.4	7.1 ± 0.2	6.2 ± 0.1	71.1	50.2 ± 0.5
T3	11.2 ± 0.6	32.3 ± 1.4	14.4 ± 0.3	7.2 ± 0.2	6.3 ± 0.3	71.4	52.0 ± 0.1
T4	11.4 ± 0.6	32.5 ± 1.1	14.7 ± 0.4	7.5 ± 0.1	6.2 ± 0.3	72.3	53.3 ± 0.7
T5	11.2 ± 0.5	32.2 ± 0.3	14.9 ± 1.1	7.5 ± 0.1	6.4 ± 0.2	72.2	53.9 ± 0.1
T6	11.6 ± 0.6	32.5 ± 0.9	14.8 ± 0.7	7.3 ± 0.3	6.3 ± 0.4	72.5	53.2 ± 0.6
T7	11.5 ± 1.1	32.2 ± 0.7	14.7 ± 0.5	7.5 ± 0.2	6.3 ± 0.3	72.2	53.0 ± 0.9

Table 3: Mean (± SE) primary fold heights (µm) of magnum in White Leghorn layers fed different levels of tryptophan and crude protein at 45th week of age

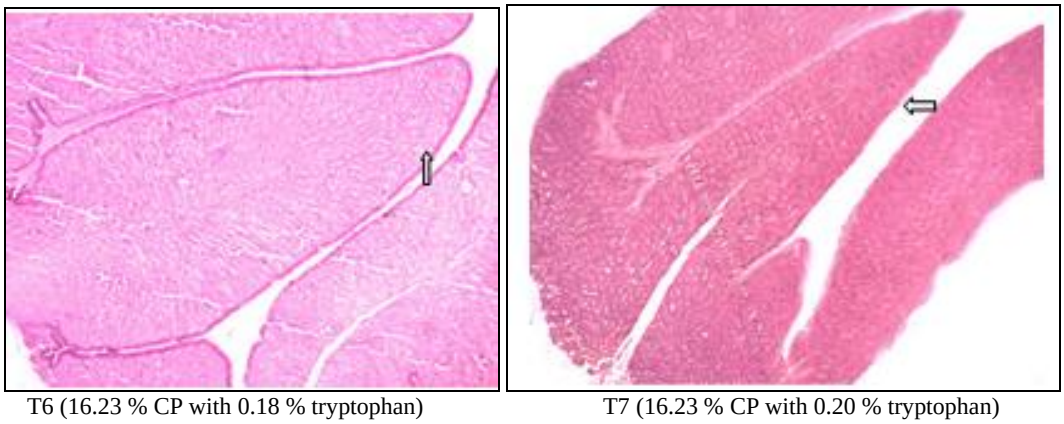
Treatment	Magnum
T1	3182.00 ± 5.00
T2	3150.5 0± 3.70
T3	3159.70 ± 4.80
T4	3198.4 0± 5.00
T5	3201.8 0± 2.60
T6	3186.10 ± 4.70
T7	3155.70 ± 4.20



T1 (17.00 % CP with 0.165 % tryptophan) T2 (16.23 % CP with 0.153 % tryptophan)



T4 (17.00 % CP with 0.18 % tryptophan) T5 (17.00 % CP with 0.20 % tryptophan)



T6 (16.23 % CP with 0.18 % tryptophan) T7 (16.23 % CP with 0.20 % tryptophan)

Plate 1: Primary fold heights (µm) of magnum in White Leghorn layers fed different levels of tryptophan and crude protein at the age of 45 weeks (H&E x 40)

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