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Effect of Dietary Tryptophan Supplementation on Uterine Morphology in Layer Chicken

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A B S T R A C T

The study was conducted to ascertain the effect of supplemental tryptophan on uterine 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, oviducts from six birds per treatment were dissected out and gross morphometrical parameters like length and weight were recorded. For histological studies, after tissue preparation and staining with H&E, histological layer of uterus was studied. Our data analysis indicated that the total weight and length of various segments of oviduct did not differ by tryptophan supplementation. The histological layers of uterus were also not altered by tryptophan supplementation.

Introduction

Reproduction in poultry is completely different from other farm animal species. In poultry, there are two principle reproductive organs; ovary and oviduct; which participate in the production of eggs in laying hen. The oviduct of chicken is an egg producing organ, which convey the ovum to the cloaca and it successively adds the albumin from the glands of magnum. Finally, shell membrane is added from isthmus, the hard calcite shells and shell

pigments from uterus and the bloom or cuticle from the gland of vagina. Thus, oviduct play vital role in the assembly of egg components after receiving ova from ovary.

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 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 (Lima *et al.,* 2012). The present investigation was conducted to study the anatomy and the histological features of uterus 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 at Veterinary College and Research Institute, Namakkal, Tamil Nadu.

At the age of 18 weeks, after two weeks of adaptation the layer birds were divided into seven treatments with five replicates per treatment and each replicate had 10 birds. The layers were reared in cages in gable roofed open sided, elevated platform house. All the birds were provided with uniform cage floor, feeder and water space and 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.

Diet I (T1) was formulated with 17 per cent protein (normal) as followed in commercial layer farms with the digestible tryptophan of 0.165 %. Diet II (T2) was formulated with 0.153 % digestible tryptophan, meeting requirements of other essential amino acids as per breeder's specification with low protein (16.23 %).

Diet III (T3) was formulated to meet out the difference in digestible tryptophan between diet I and II by supplementing digestible tryptophan at the level of 0.012 % to basal diet II. Diet IV (T4) and V (T5) were formulated by supplementing 0.015 % and 0.035 % tryptophan to diet I to attain 0.18% and 0.20 % digestible tryptophan levels respectively. Diet VI (T6) and VII (T7) were formulated by supplementing 0.027 % and 0.047 % tryptophan to diet II to attain 0.18 % and 0.20% digestible tryptophan levels respectively.

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 and stretched on a paper. The length (cm) of the oviduct was measured and weighed. The morphological parameters like total length and weight of oviduct, individual length of various segments of oviduct were recorded by caliper device. For histological observations, uterus of the oviduct was separated by incision and fixed in Bouins

fluid and processed for routine microtome. After tissue preparation and staining with H and E as per Bancroft and Stevens (1996), histological layers of uterus such as tunica mucosa, submucosa and muscularis were recognized and the primary fold length of tunica mucosa were measured using micrometry method. Photographs of the prepared slides were taken with microscope equipped with a camera (Zeiss primostar, Germany with Axiocam ERC5S camera).

Results and Discussion

The effect of supplementation of tryptophan on morphometrical parameters of oviduct and primary fold length of uterus 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 Mohammadpour *et al.,* (2012) in 12-18 months and Mishra *et al.,* (2014) 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 uterus of layer chicken showed normal histological structures (Plate 1). The mean lengths of primary folds (µm) of uterus in our study were in normal range of 1367.8 ± 3.0 to 1396.7 ± 2.6 and our findings are in agreement to Mohammadpour *et al.,* (2012) 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 uterus.

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
SodaBicarb (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

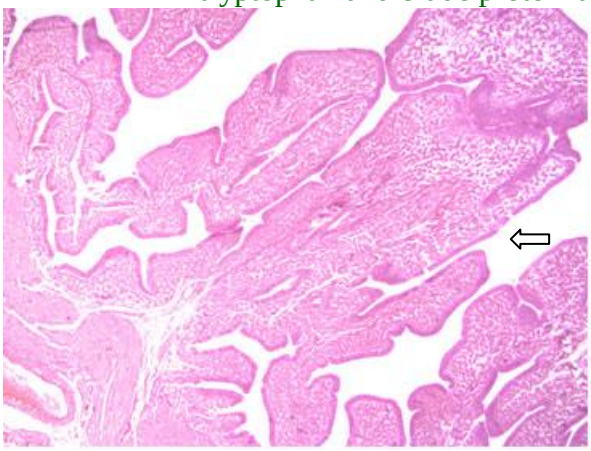
Table.3 Mean (± SE) primary fold heights (µm) of uterus in White Leghorn layers fed different levels of tryptophan and crude protein at 45th week of age

Treatment	Uterus
T1	1385.70 ± 4.80
T2	1367.80 ± 3.00
T3	1377.80 ± 4.20
T4	1394.90 ± 2.60
T5	1396.70 ± 2.60
T6	1375.20 ± 4.40
T7	1376.50 ± 1.50

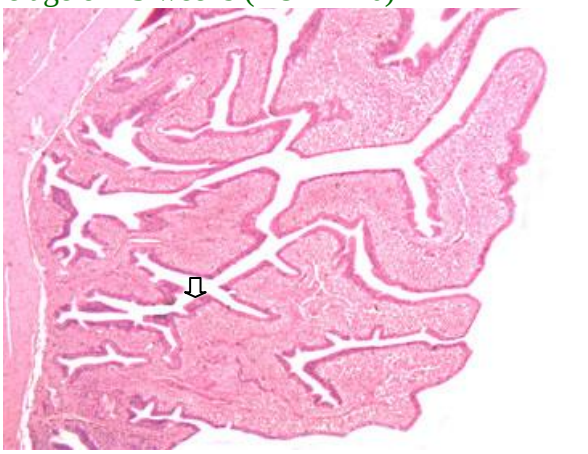
The experimental groups and their diets are as follows

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

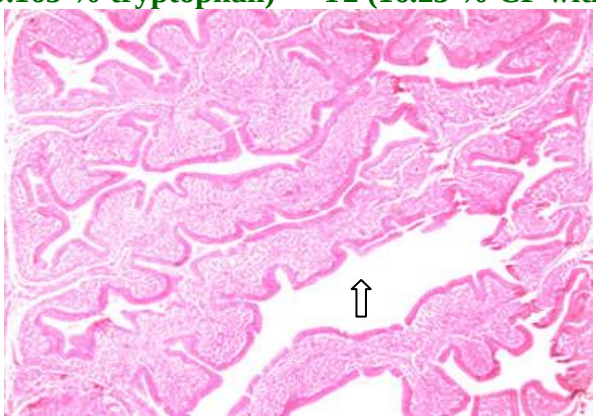
Plate.1 Primary fold heights (µm) of uterus in White Leghorn layers fed different levels of tryptophan and crude protein at the age of 45 weeks (H&E x 40)



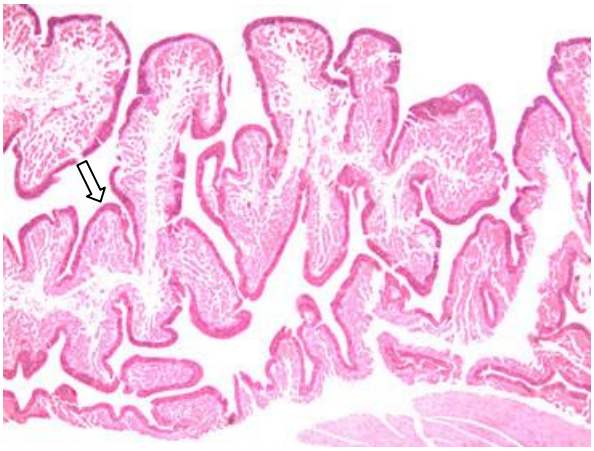
T1 (17.00 % CP with 0.165 % tryptophan)



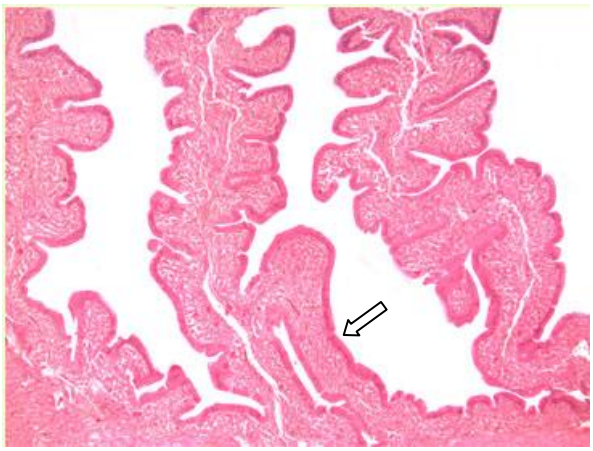
T2 (16.23 % CP with 0.153 % tryptophan)



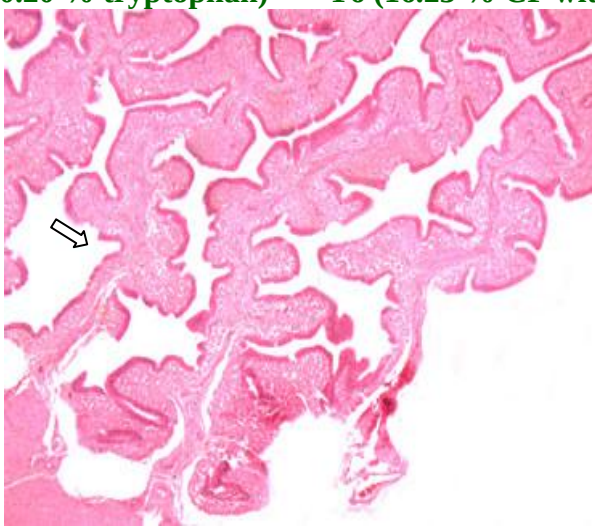
T4 (17.00 % CP with 0.18 % tryptophan)



T5 (17.00 % CP with 0.20 % tryptophan)



T6 (16.23 % CP with 0.18 % tryptophan)



T7 (16.23 % CP with 0.20 % tryptophan)

Our findings were contrary to Lima *et al.*, (2012) who observed increased uterine folds in laying hens supplemented with tryptophan at 0.183, 0.199 and 0.215 %.

The histomorphological results indicated that tryptophan supplementation did not cause any histological changes in uterus of layer hens.

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