

Original Research Article

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Effect of Dietary Tryptophan Supplementation on Blood Biochemical Parameters in Layer Chicken

Manju G. Preedaa^{1*}, P. Selvaraj¹, P. Visha¹, M.R. Purushothaman² and N. Murali³

¹Department of Veterinary Physiology, ²Department of Animal Nutrition, ³Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), India

**Corresponding author*

ABSTRACT

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The study was conducted to determine the effect of dietary supplemental tryptophan on the levels of blood biochemical parameters namely glucose, total cholesterol, total protein, albumin and globulin. 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. Blood was collected from the birds at monthly intervals and plasma was separated to analyse glucose, total cholesterol, total protein and albumin using UV spectrophotometer. The results of the study indicate that increasing tryptophan levels at 0.18 and 0.20 % had significantly ($P<0.01$) decreased plasma cholesterol and increased total protein and albumin levels, whereas plasma glucose levels did not vary due to tryptophan levels.

Introduction

Indian poultry industry has been registering an annual growth rate at around 8-10 per cent over the last decade. India ranks third in the world with annual egg production of 74752 millions. Modern commercial layers are able to produce more than 310 eggs per hen. Thus there is a tremendous opportunity for further growth of the industry. Research on poultry have shown that supplementation with industrial amino acids (AA) on low protein diets is an effective method to reduce feed costs and minimize nitrogen excretion.

Birds rely on continuous protein supply throughout life for maintenance, growth and reproduction. Modern laying hens are able to achieve high indexes of productivity. However, for this, it is essential that they receive diets with adequate levels of amino acids. Essential amino acids have an important role in the maintenance, growth, egg production and serve as precursors of various enzymes and hormones essential in the metabolism. Thus, the amino acid levels of diets should be precisely balanced and therefore it is important to establish the ideal

ratios between essential amino acids and lysine rather than determining the absolute requirement of the birds for each amino acid.

Tryptophan is considered to be the third limiting amino acid in diets based on corn and soybean meal for laying hens. However, few studies have been conducted to determine the requirement of digestible tryptophan and its ideal ratio with digestible lysine in diets for laying hens. Tryptophan plays a significant role in low protein laying hen rations. Supplemented synthetic L-tryptophan is 99.3 % digestible (Russell and Harms, 1999; Harms and Russell, 2000; Peganova *et al.*, 2003; Deponti *et al.*, 2007).

Among the essential amino acids that commonly constitute proteins, tryptophan is found in the lowest proportion in plasma or blood. It has relatively low tissue storage and the overall tryptophan concentration in the body is the lowest among all amino acids (Reilly *et al.*, 1997). Despite its relatively low concentration in tissues, tryptophan has multiple metabolic functions. This plays an important role in protein synthesis and acts as precursor for a variety of biologically active compounds such as serotonin, niacin, melatonin, anthranilic acid, tryptamine and kynurenic acid. In addition, tryptophan is a precursor for coenzymes NAD, NADP that could replace niacin as an essential nutrient (Shin *et al.*, 1991).

High levels of tryptophan had positive effects on systemic immune response and growth performance in birds (Emadi *et al.*, 2010). Tryptophan was also important to alleviate the depression of weight gain and feed intake caused by niacin deficiency in poultry (Xie *et al.*, 2014).

Therefore the aim of this study was to investigate the effects of dietary tryptophan as one of the essential amino acids on blood

biochemical indices during different periods of laying hen.

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 the Poultry Farm Complex, Department of Poultry Science, 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 %).

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

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 and proximate analysis of nutrients done as per AOAC (1995) are presented in table 1.

Blood (2 ml) was collected from wing vein using 24 G needles in sodium fluoride vacutainer tubes from 42 birds (six birds per treatment) at monthly intervals.

The collected blood was immediately centrifuged for 10 min at 2,000 rpm and the separated plasma was stored at -20° C for further biochemical analysis.

The blood biochemicals were analyzed with UV-VIS double beam spectrophotometer (SYSTRONICS, Model 2202, India) using commercial kits (Span diagnostics Ltd., India).

Total cholesterol (Wybenga *et al.*, 1970), glucose (Tietz, 1976), total protein (Lowry *et al.*, 1951), albumin (Johnson *et al.*, 1999) and globulin were determined in the plasma samples as per the manufacture's protocol.

Statistical analysis

The data collected were analysed using SPSS® 20.0 software package. Post hoc analysis was done by Duncan's multiple descriptive significant difference. All the statistical procedures were performed based upon Snedecor and Cochran (1994).

Results and Discussion

The influence of digestible tryptophan at different levels on the blood biochemical constituents of layer chicken from 27 to 43 weeks are presented in tables 2–6.

The mean plasma glucose concentration did not show any significant variation among the treatment groups, but exhibited a non-significant increase from 27th to 31st week and these increased levels were maintained till the end of experiment (Table 2).

The results of the present study concurred with that of Gogary and Azzam, (2014) at 0.25, 0.5, 0.75 and 1.0 g/kg of dietary tryptophan levels and Rojas *et al.*, (2015) at 0.16 and 0.18 % tryptophan who recorded tryptophan had no significant change in the blood glucose level. Supplementation of

tryptophan had significant changes in the mean plasma cholesterol concentration (Table 3) of layer chicken from 27 to 43 weeks. The mean plasma cholesterol level was significantly ($P < 0.01$) low in tryptophan supplemented groups to 0.18 % and 0.20 % levels in normal protein (T4 and T5) and low protein (T6 and T7) diets respectively, when compared to diets containing normal protein with 0.165 % tryptophan (T1) and low protein with 0.153 % (T2) and 0.165 % (T3) tryptophan throughout the entire experimental period. The plasma cholesterol levels did not vary between tryptophan supplemented groups in both normal (T4 and T5) and low (T6 and T7) protein diets with lowest level exhibited by T5 group. Similarly, the plasma cholesterol levels did not vary between other

groups (T1, T2 and T3) with highest level exhibited by T2 group.

The plasma cholesterol level reduced uniformly by about 20 % in 0.18 % and 0.20 % tryptophan supplementation in normal protein diet than control group.

Similar to the present study, reduction in the levels of plasma total cholesterol by supplemental tryptophan were observed in broilers by Corza *et al.*, (2010) (138 to 125 mg/dl at 2.3 g/kg), Emadi *et al.*, (2010) (250 to 181 mg/dl at 0.13 g/kg), Gogary and Azzam (2014) (233 to 189 mg/dl at 0.25 g/kg) and in White Plymouth Chicken by Moneva *et al.*, (2011) (140 to 120 mg/dl at 5 g/kg).

Table.1 Ingredients and nutrient composition (%) of layer diet fed different levels of tryptophan and crude protein

Ingredients	Layer diet	
	Diet I (normal protein)	Diet II (low protein)
Maize %	49.6	52.2
Deoiled rice bran %	15.0	15.0
Sunflower oil cake %	5.1	4.3
Soyabean meal %	16.5	14.7
Fish meal %	3.1	3.2
Di calcium phosphate %	0.56	0.58
Calcite %	4.0	4.0
Shell grit %	5.7	5.7
DL-Methionine (g/100kg)	141	165
Lysine (g/100kg)	0	34
L-Threonine (g/100kg)	34	62
SodaBicarb (g/100kg)	55	61
Crude protein	17.03	16.60
Crude fibre	6.07	6.31
Calcium	5.00	4.50
Ether extract	3.00	3.04
Available phosphorus*	0.64	0.63
Lysine*	0.73	0.70
Methionine*	0.40	0.42
Metabolizable Energy* (kcal/kg)	2550	2570

* Calculated values

Table.2 Mean (\pm SE) plasma glucose (mg/dl) of White Leghorn layers fed different levels of tryptophan and crude protein during 27 to 43 weeks of age

Treatment	Age in weeks				
	27	31	35	39	43
T1	200.30 \pm 6.86	208.33 \pm 1.94	208.98 \pm 4.10	209.12 \pm 0.70	209.19 \pm 1.98
T2	200.16 \pm 7.84	207.85 \pm 1.92	208.70 \pm 7.44	208.67 \pm 1.34	208.71 \pm 3.14
T3	200.78 \pm 4.05	208.41 \pm 1.77	208.79 \pm 6.55	208.64 \pm 1.14	208.91 \pm 2.92
T4	205.27 \pm 8.01	212.48 \pm 1.04	213.57 \pm 1.34	213.22 \pm 2.61	214.76 \pm 0.97
T5	206.67 \pm 7.15	213.60 \pm 1.32	213.94 \pm 1.58	213.79 \pm 1.35	214.90 \pm 0.77
T6	206.12 \pm 6.75	211.43 \pm 1.54	212.68 \pm 1.78	213.50 \pm 2.26	214.42 \pm 2.02
T7	206.36 \pm 8.89	212.86 \pm 2.01	212.54 \pm 2.56	213.73 \pm 2.17	214.83 \pm 3.48

Table.3 Mean (\pm SE) plasma cholesterol (mg/dl) of White Leghorn layers fed different levels of tryptophan and crude protein during 27 to 43 weeks of age

Treatment	Age in weeks				
	27	31	35	39	43
T1	159.88 ^b \pm 4.89	154.35 ^b \pm 2.92	149.74 ^b \pm 2.90	146.06 ^b \pm 2.93	154.77 ^b \pm 3.39
T2	160.78 ^b \pm 2.89	155.22 ^b \pm 3.68	150.93 ^b \pm 2.86	146.47 ^b \pm 2.71	155.2 ^b \pm 2.82
T3	159.73 ^b \pm 3.80	150.87 ^b \pm 3.73	150.00 ^b \pm 4.50	145.78 ^b \pm 3.70	154.48 ^b \pm 3.72
T4	142.99 ^a \pm 4.10	130.58 ^a \pm 3.18	134.79 ^a \pm 6.27	130.43 ^a \pm 2.71	128.78 ^a \pm 11.63
T5	139.67 ^a \pm 3.21	130.00 ^a \pm 3.29	131.61 ^a \pm 2.70	128.63 ^a \pm 3.60	128.63 ^a \pm 4.94
T6	145.55 ^a \pm 3.51	132.90 ^a \pm 3.12	135.19 ^a \pm 5.79	132.37 ^a \pm 3.03	131.42 ^a \pm 6.29
T7	145.10 ^a \pm 2.09	134.30 ^a \pm 3.14	134.92 ^a \pm 3.25	132.64 ^a \pm 3.02	131.86 ^a \pm 5.57

Means bearing different superscript within the column differ significantly (P<0.01)

Table.4 Mean (\pm SE) plasma total protein (g/dl) of White Leghorn layers fed different levels of tryptophan and crude protein during 27 to 43 weeks of age

Treatment	Age in weeks				
	27	31	35	39	43
T1	3.63 ^a \pm 0.06	3.67 ^a \pm 0.09	3.86 ^a \pm 0.12	3.86 ^a \pm 0.06	3.83 ^a \pm 0.17
T2	3.36 ^a \pm 0.13	3.33 ^a \pm 0.14	3.59 ^a \pm 0.14	3.60 ^a \pm 0.07	3.61 ^a \pm 0.13
T3	3.60 ^a \pm 0.10	3.64 ^a \pm 0.15	3.81 ^a \pm 0.19	3.82 ^a \pm 0.11	3.85 ^a \pm 0.12
T4	4.23 ^b \pm 0.10	4.21 ^b \pm 0.07	4.43 ^b \pm 0.09	4.44 ^b \pm 0.14	4.50 ^b \pm 0.07
T5	4.43 ^b \pm 0.21	4.43 ^b \pm 0.16	4.54 ^b \pm 0.12	4.55 ^b \pm 0.13	4.59 ^b \pm 0.10
T6	4.19 ^b \pm 0.14	4.20 ^b \pm 0.18	4.38 ^b \pm 0.07	4.4 ^b \pm 0.08	4.43 ^b \pm 0.10
T7	4.22 ^b \pm 0.11	4.22 ^b \pm 0.19	4.42 ^b \pm 0.07	4.44 ^b \pm 0.09	4.46 ^b \pm 0.05

Means bearing different superscript within the column differ significantly (P<0.01)

Table.5 Mean (\pm SE) plasma albumin (g/dl) of White Leghorn layers fed different levels of tryptophan and crude protein during 27 to 43 weeks of age

Treatment	Age in weeks				
	27	31	35	39	43
T1	2.37 ^a \pm 0.08	2.27 ^a \pm 0.04	2.29 ^a \pm 0.03	2.30 ^a \pm 0.04	2.3 ^a \pm 0.07
T2	2.30 ^a \pm 0.05	2.2 ^a \pm 0.03	2.18 ^a \pm 0.04	2.24 ^a \pm 0.04	2.24 ^a \pm 0.04
T3	2.36 ^a \pm 0.03	2.24 ^a \pm 0.05	2.26 ^a \pm 0.03	2.28 ^a \pm 0.03	2.26 ^a \pm 0.06
T4	2.80 ^b \pm 0.06	2.70 ^b \pm 0.04	2.77 ^b \pm 0.05	2.79 ^b \pm 0.03	2.78 ^b \pm 0.04
T5	2.92 ^b \pm 0.04	2.71 ^b \pm 0.05	2.86 ^b \pm 0.05	2.80 ^b \pm 0.02	2.88 ^b \pm 0.06
T6	2.77 ^b \pm 0.04	2.69 ^b \pm 0.03	2.74 ^b \pm 0.06	2.76 ^b \pm 0.03	2.77 ^b \pm 0.02
T7	2.79 ^b \pm 0.05	2.69 ^b \pm 0.04	2.76 ^b \pm 0.03	2.77 ^b \pm 0.02	2.78 ^b \pm 0.03

Means bearing different superscript within the column differ significantly (P<0.01)

Table.6 Mean (\pm SE) plasma globulin (g/dl) of White Leghorn layers fed different levels of tryptophan and crude protein during 27 to 43 weeks of age

Treatment	Age in weeks				
	27	31	35	39	43
T1	1.25 ^{ab} \pm 0.09	1.40 ^{ab} \pm 0.09	1.57 \pm 0.09	1.56 ^{ab} \pm 0.02	1.53 ^{ab} \pm 0.12
T2	1.06 ^a \pm 0.10	1.13 ^a \pm 0.12	1.42 \pm 0.13	1.37 ^a \pm 0.04	1.37 ^a \pm 0.17
T3	1.24 ^{ab} \pm 0.08	1.41 ^{ab} \pm 0.10	1.55 \pm 0.17	1.54 ^{ab} \pm 0.08	1.59 ^{ab} \pm 0.07
T4	1.42 ^{ab} \pm 0.06	1.51 ^{ab} \pm 0.07	1.66 \pm 0.06	1.65 ^b \pm 0.11	1.72 ^b \pm 0.04
T5	1.50 ^b \pm 0.20	1.72 ^b \pm 0.14	1.68 \pm 0.09	1.74 ^b \pm 0.10	1.71 ^b \pm 0.06
T6	1.42 ^{ab} \pm 0.13	1.51 ^{ab} \pm 0.16	1.64 \pm 0.02	1.64 ^b \pm 0.06	1.65 ^b \pm 0.08
T7	1.42 ^{ab} \pm 0.11	1.52 ^{ab} \pm 0.16	1.65 \pm 0.04	1.67 ^b \pm 0.08	1.68 ^b \pm 0.02

Means bearing different superscript within the column differ significantly (P<0.05)

The reduction in total cholesterol level in tryptophan supplemented groups may be due decreased lipolysis and reduction in plasma free fatty acid by niacin yielded from supplemental tryptophan. Niacin also inhibits diacylglycerol acyltransferase-2 (DGAT2) resulting in decreased triglyceride synthesis. Decrease in cholesterol may be due to reduced stress by tryptophan supplementation as plasma cholesterol is a promising stress indicator (Puvadolpirod and Thaxton, 2000).

The plasma total protein and albumin levels were significantly (P<0.01) high in tryptophan supplemented groups of both normal (T4 and T5) and low (T6 and T7) protein diets than other groups (T1, T2 and T3) throughout the entire period (Tables 4 and 5).

During 27 and 31 weeks, the plasma globulin levels showed significantly (P<0.05) increased values at higher supplementation of tryptophan (0.20 %) in normal protein group (T5) than all other treatments. The plasma globulin levels did not vary among the treatment groups during 35th week. During the later period (39 and 43 weeks), the plasma globulin level was lowest in T2 (low protein and 0.153 % tryptophan) than all other groups and it was similar in T1, T3, T4, T5, T6 and T7 groups (Table 6). Similar effects of increased plasma total protein and albumin by tryptophan supplementation were reported by

Emadi *et al.*, (2010) in broiler chicken at 0.07, 0.13, 0.20, g/kg, in 40 weeks old Babcock Brown layers at 0.4 g/kg of diet (Dong *et al.*, 2012), at 0.22 and 0.30 % tryptophan in Goslings (Pan *et al.*, 2013).

The increase in plasma total protein and albumin in tryptophan supplemented groups might be due to tryptophan induced increased serum IGF-I which enhances the cellular absorption of amino acids (Jacob *et al.*, 1989). Further, tryptophan inhibits the expression of cathepsin B and 20S protease and plasma cortisol secretion thus inhibiting protein degradation (Simmons *et al.*, 1984).

Dietary nutrient levels can also influence amino acid balance and plasma total protein levels which tends to increase when birds are raised with rich nutrition (Choi *et al.*, 2005). Amino acids affect the synthesis of immunoglobulin. Deficiency of tryptophan decreased antibody production in rats (Gershoff *et al.*, 1968).

In conclusion, as protein or amino acid levels in feed for poultry make up a significant portion of feed cost and are determinants of batch productivity, they directly interfere with the profitability of the poultry enterprise. Currently, protein sources in layer diets have been partially replaced by industrial amino acids such as tryptophan. It could be inferred from the present study that in birds fed low

protein diets, tryptophan maintained the normal levels of blood biochemical and enhanced the egg production.

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