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Effect of dietary tryptophan on plasma growth hormone and thyroid hormone in broiler chicks

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ABSTRACT

The goal of this experiment was to clarify the effects of dietary tryptophan (Trp) on the growth hormone (GH), T_3 and T_4 in broiler chicks in 0-3 wk. In this study different levels of dietary Trp (0.12%, 0.16%, 0.20%, 0.24% and 0.28%) were used for first three weeks in male broiler chicks. Animal randomly divided into five treatments with four replicates. Blood samples were collected in 11 and 21 days. Plasma concentrations of GH, $T_{3,}$ and T4 were measured using ELISA kits. The results showed that reduction in dietary Trp significantly increase concentrations of plasma GH and T_3 in 11 day and 21day (P<0.05). Increase of dietary Trp compared with NRC recommended level (0.20%Trp) decrease a little level of GH, T_3 in two period (P>0.05). Dietary Trp had no significant effect on T_4 in 11 day (P>0.05) but reduction of dietary Trp level significantly decrease of T_4 level in broiler chicks. Therefore, we conclude that dietary Trp levels in chicks inversely affected GH,T_3 levels in 11, 21dayes and only significantly affected T_4 in 21day.

Key words: Tryptophan, Growth hormone, Thyroid hormone, Broiler

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INTRODUCTION

Tryptophan is one of eight essential amino acids that must be obtained from diet (2, 18). For all amino acids, including L-tryptophan, only the L isomer is used in protein synthesis and can pass across the blood- brain barrier (18, 21). Its concentration in organisms is among the lowest of all amino acids (8) and it has relatively low tissue storage also Trp can be used for the feed additive which exerts the stress reducing effect in domestic animals (20). Trp is the primary precursor of serotonin (11, 15). It has a broad impact as a neurotransmitter and neuromodulator (18). Because serotonin does not cross the blood-brain barriers, its effects within the central nervous system depend on the transfer of tryptophan across that barrier, so the production of serotonin in the brain is limited by the availability of its plasma dietary amino acid precursor tryptophan(20, 21).

Several studies have shown that serotonin and tryptophan (its biochemical precursor) be involved in animal growth hormone (GH) and thyroid hormones. Infusion of 5-HT (5- hydroxytryptamin) significantly increased the plasma concentration of the T3, T4 and GH in goat (13) and cattle (12). Injecting L-Trp enhanced mean plasma GH levels in rat (1), but administrationof Para-Chlorophenylalanine (*p*CPA) led to a significant rise in circulating GH concentrations in male domestic fowl treated with centrally active agents (10). Moreover in chicks, low-protein diet increase GH levels and T3, T4 levels increased and decreased, respectively (3, 15).

The level of Trp recommended for broiler chickens by the NRC (1994), from 0 to 3 wk of age, is 0.20% of the diet. This study was conducted to evaluate the effects of dietary L-Trp levels on GH, T_3, T_4 in broiler chicks in first 3wk(16).

MATERIALS AND METHODS

Two hundred twenty one-day- old Ross 308 male broiler chicks were obtained from a commercial source. There were five treatments with 4 replicates and 11 chicks were randomly placed on fresh wood shaving in floor pens of an open-sided house with controlled standard temperature, humidity, ventilation. Photo period was 24 h of light(2.5 Watt m^{-2}) until 21 days of age. The temperature of the room was 32°C in the first week and was then reduced by 3°C weekly. Feed and water were provided ad libitum. The experiment was conducted for first 3 wk.

Dietary treatments:
Experiment designed with five levels of Trp (0.12, 0.16, 0.20, 0.24, and 0.28 percent in diet). The basal diets (Table 1) were based on Corn, Corn gluten, gelatin by-product, poultry fat, DL-Met, L-Lys, L-Arg and Thr (23% cp and 3.34 kcal of metabolizable energy/g of diet). At 11, 21 days of age, one chicken from each replicate of each treatment were selected for blood sampling from using sterile and heparinized syringes.

Table1: Composition of the basal diet

Composition of the basal diet	
Corn, grain	70.79
Corn gluten meal	17.44
Gelatin by-product	5.00
Poultry fat	2.00
Defluorinate phosphate	1.92
Limestone	0.71
L-Lys	0.75
L-ArgHCl	0.34
L-Thr	0.19
DL-Met	0.08
Common salt	0.40
Vitamin premix[1]	0.25
Mineral premix[2]	0.08
Total	100.00
Composition from table values[3]	
Crude protein (%)	23.00
Metabolizable energy (kcal/g	3.34
Calcium (%)	0.90
Available phosphorous (%	0.45
Trp (%) [4]	0.09

[1]Vitamin premix provided the following per kilogram: Vitamin A, 5,500 IU from all trans-retinyl acetate, cholecalciferol, 1,100 IU; vitaminE, 11 IU from all-rac-α-tocopherol acetate; riboflavin, 4.5 mg; Ca
pantothenate, 12 mg; nicotinic acid, 44 mg; choline Cl, 220 mg; vitaminB12, 6.6 µg; Vitamin B6, 2.2 mg;menadione, 1.1mg(as menadione sodium
bisulfate); folic acid, 0.55 mg; d-biotin, 0.11 mg; thiamine, 1.1 mg (asthiaminemononitrate); ethoxyquin, 125 mg.
[2]Trace mineral premix provided the following in milligrams perkilogram of diet: Mn, 60; Zn, 50; Fe, 30; Cu, 5; I, 1.5; Se, 1.0.
[3] NRC (1994).
[4]analyzed Trp values are 0.12 %

GH, T_3, T4 hormones assays:
Blood samples were kept at 4°C until centrifugation, then centrifuged at 1:800×g and plasma were stored at -20°C until assayed for GH, T_3 ,T_4 hormones by ELISA system (AWAKNESS technology Inc, USA) including ELISA plate washer (state fax 2006), ELISA kit for growth hormone : USCN life, Cat. N: E0044Ga for T_3 hormone CUSABIO Cat. N. CSB-E13270C and for T_4 hormone CUSABIO Cat. N. CSB-E15787C.

Statistical analysis:
Data analysis was performed by using general linear model procedures of sas (SAS Institute, 2001). Significant differences ($p<0.05$) among treatment means were determined using Duncan's new multiple range test(7).

RESULTS

Mean of the GH, T_3 and T_4 plasma hormones concentrations in 11 and 21 days of age are presented in Table 1 and Table 2 respectively. Mean concentrations of plasma GH were affected significantly ($p<0.05$) by decrease of dietary Trp levels and were high in 0.12% Trp in the two period and the lowest concentrations of plasma GH were observed in broiler fed on 0.28%Trp in the two period. Increase of dietary Trp compared with control which received 0.20% dietary Trp (NRC 1994,) had no significant effect on concentration of plasma GH in 11 and 21 days($p>0.05$). Mea concentrations of plasma T_3 T_4 show that reduction in dietary Trp led to significantly increase plasma T_3 levels ($p<0.05$) in broilers chicks in 11 and 21 days. Lowest of T_3 levels were observed on 0.28%Trp in 11 day and on 0.24 %Trp in 21 day and Table1 shows that dietary Trp did not significantly affected ($p>0.05$) plasma T_4 levels in 11 day, and mean concentrations of plasma T_4 by reduction of dietary Trp significantly affectedat 21 d of age($p<0.05$) but did not significantly affected in 11 day($p>0.05$).

Table2: Effect of level of dietary tryptophan on plasma growth hormone,T_3 and T_4concentrations in 11 day

Treatment	**T_3** (ng/dlit)	**T_4** (µg/dlit)	**GH**(ng/ml)
0.12 Trp%	257.43^a	4.11	2.9^a
0.16 Trp%	216.80^{ab}	4.16	2.36^b
0.20 Trp%	212.97^{ab}	4.76	2.26^b
0.24 Trp%	174.90^{ab}	4.81	2.06^b
0.28 Trp%	149.80^b	4.38	1.93^b
SEM	26.63	0.5	0.14
P value	0.117	0.786	0.0079

Means of control groups with the same letter are not significantly ($p<0.05$).

Table3: Effect of level of dietary tryptophan on plasma growth hormone,T_3 and T_4cocentrations in 21 day

Treatment	**T_3**(ng/dlit)	**T_4**(µg/dlit)	**GH**(ng/ml)
0.12 Trp%	202.42^a	2.98^c	2.73^a
0.16 Trp%	154.15^b	3.94^b	2.26^b
0.20 Trp%	91.42^c	4.27^{ab}	2.48^{ab}
0.24 Trp%	82.68^c	4.80^a	2.31^b
0.28 Trp%	94.18^c	4.39^{ab}	2.20^b
SEM	16.09	0.2	0.11
p value	0.0001	<0.0001	0.02

Means of control groups with the same letter are not significantly ($p<0.05$).

DISCUSSION

The present results show that dietary Trp affect, in a dose-dependent manner, on the levels of GH, T_3, and T_4 in broiler chicks at the first three weeks. This effect could be due to the function of tryptophan as a precursor of the neurotransmitter of serotonin(14). Experimental research has shown that L-tryptophan's role in brain serotonin synthesis is an important factor involved in mood, behavior, and cognition(14, 19, 22). Tryptophan also exerts effects on other neurotransmitters and CNS compounds through serotonin synthesis. Tryptophan is also thought to be involved in modulation of the endocrine system (14, 18). Injecting L-tryptophan or a serotonin receptor agonist pyrazine in male rats bearing right atrial cannulae significantly enhanced mean plasma GH levels and support the notion that treatments which increase serotonin receptor stimulation enhance or induce pulsatile GH secretion(1). Khazali et al. indicated that Infusions of serotonin increase the mean plasma concentration of GH, T3, T4 in the Sanan goats (13). Kasuya et al. showed that Concentrations of 5-HT in the cerebrospinal fluid were increased by Trp infusion in cattle and significantly increased GH release and The direct effect of Trp on GH release from the dispersed anterior pituitary cells did not stimulate GH release from the bovine pituitary cells so These results suggest that Trp induced GH release via the activation of serotonergic neurons in cattle (12).Denbow et al.reported that supplemental Trp increased brain serotonin concentrations in young turkeys (6). J.Rabii and et al. showed that in the young male domestic fowl serotonin has an inhibitory role in the regulation of GH secretion (17). T. R. Hall reported thatSerotonin and its agonist quipazine inhibited the release of GH in broiler fowl (9).Unlike mammals serotonin appears to suppress GH secretion by reducing pituitary sensitivity to releasing factors and by reducing hypothalamic GH releasing activity(10).

This study showed that dietary Trp affect T_3 T_4 in broiler chicks and reduction in dietary Trp led to significantly increase plasma T_3 levels also reduced plasma T_4but,this decreasewas notsignificantin11d. Similar finding were reported by L.B.Carew et al. that showed plasma T_3 was elevated in tryptophan –deficient chicks and plasma T_4 was also reduced (4). Plasma growth hormone levels are higher in chickens reared on a low protein diet, indicating a causal relationship between growth hormone secretion and protein efficiency(20) and plasma T3 and T4 levels are well known to increase and decrease, respectively, with decreasing protein content(3, 20). It was the same effect that was observed in theTrp deficient chicks on the other hand , several studies have shown that low intakes of dietary protein influence thyroid function and plasma T3 is often elevated in protein-deficient chicks and accompanied frequently by depressions in plasma T4 (2, 3, 15). Increases in activity of deiodinase enzymes resulting in an augmented conversion of T4 to T3 could also readily explain the effect of certain amino acid deficiencies on circulating levels of thyroid hormones. However, we have found no change in the activities of hepatic or renal microsomal 5′-deiodinase in protein deficient chicks (3).

In mammals, different types of iodothyroninedeiodinating enzymes (type I, II and III) have been characterized. It has been shown that in chicken liver both typ I and type III activity are present (5). Darras et al. showed that GH has no effect at all on the amount of type I enzyme (catalyzing T_4deiodination to T_3) but acutely decreased the amount of type III enzyme (catalyzing T_3deiodination) and GH-induced showed an elevation on plasma T_3 was not due to an increased T_3 production, but was the result of a decreased T_3 breakdown (5). However, the results described here suggest that dietary tryptophan influence thyroid hormone levels possibly by change the GH level in chicks.

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