


## ORIGINAL ARTICLE

# Effect of dietary tryptophan supplementation on growth performance, immune response and anti-oxidant status of broiler chickens from 7 to 21 days

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## Abstract

**Background:** This study was conducted to investigate the optimum dietary level of tryptophan (Trp) supplementation at which broiler chickens have better growth with efficient immune system and anti-oxidant status.

**Method:** One hundred and twenty ( $n = 120$ ) 1-day-old broiler chicks were fed a common commercial diet from days 1 to 7. On day 7, the chicks were randomly divided in three treatment groups, that is, Trp 0.2 [national research council (NRC) recommended level of tryptophan], Trp 0.3 (tryptophan supplemented at 0.3%) and Trp 0.5 (tryptophan supplemented at 0.5%). All the experimental diets were iso-caloric (ME; 3,000 kcal/kg) and iso-proteic (CP; 18.5%). Weekly data on feed intake and body weight gain (BWG) were recorded to calculate feed conversion ratio (FCR). On day 19, avian tuberculin was injected to note the cellular immunity. On day 21, two birds per replicate were killed to determine carcass and visceral organ weights. Blood serum samples were collected for analysis of humoral immune response against sheep red blood cells, total oxidant and anti-oxidants by spectrophotometric method.

**Results:** Feed intake, carcass and visceral organ weights remained unaffected by dietary treatments while BWG and FCR tended to improve ( $p < .05$ ) in broiler chicks fed the Trp 0.3 and the Trp 0.5 diets. Total oxidant status was also improved ( $p < .05$ ) in broiler chicks fed the Trp 0.5 diet. Likewise, broiler chicks fed the Trp 0.3 and the Trp 0.5 diets tended to have better ( $p < .05$ ) total anti-oxidant status, catalase, glutathione peroxidase, glutathione reductase and arylesterase (ARE). The overall antibodies response and IgG improved ( $p < .05$ ) by the Trp 0.3 and Trp 0.5 diets compared to control. However, IgM level remained similar across the treatment. The cellular immunity against avian tuberculin improved at 24 hr post-injection but its effect disappeared at 48 hr.

**Conclusion:** The results of present study revealed that Trp above the NRC recommended level may give better growth, immune response and anti-oxidant status in broiler chickens.

**KEYWORDS**

broiler, growth performance, immune response, oxidant and anti-oxidant status, tryptophan

## 1 | INTRODUCTION

Modern poultry production encounters various stresses especially the nutritional stress imposed by high dietary level of polyunsaturated fatty acids, mycotoxins, vitamins and minerals imbalance. Moreover, broiler chicken has been improved genetically over the years for fast growth rate, which is associated with rapid cell proliferation, consequently, the level of reactive oxygen species (ROS) increases leading to oxidative stress (Surai, 2015). The highly reactive and unstable nature of ROS has great biological concern due to their detrimental effect on cellular membranes, DNA and RNA. Thus, these ROS may create stress in the body, disturbing many metabolic and immunological pathways (Halliwell & Gutteridge, 1999).

Many studies suggested that the deficiency of dietary nutrients, especially amino acids, can impair the immune system and cellular redox status (Li, Yin, Li, Kim, & Wu, 2007). Some indispensable amino acids, in particular, methionine and tryptophan (Trp), have been reported to play important role in the prevention of oxidative stress. Tryptophan derived to 5-hydroxytryptophan (5-HT) that had role to preserve the membrane fluidity in chicken. Whereas oxidative stress puts harmful effects on membran fluidity. The 5-HT has beneficial effects on the enzymatic and non-enzymatic anti-oxidant capacity (Dong, Azzam, Rao, Yu, & Zou, 2012; Yue, Guo, & Yang, 2017).

Tryptophan deficiency leads to depressed body weight gain (BWG), lowered feed intake and poor feed conversion ratio (FCR) along with compromised antibody status (Mozhdeh et al., 2010). Tryptophan is a structural component of protein as well as a major precursor of serotonin and melatonin hormones which plays an important role in maintenance of normal physiological processes, for example, tissue synthesis, feed intake, growth performance, FCR and immunity in broiler chickens (Bai et al., 2017). Further, Trp is also involved in niacin biosynthesis in poultry (Richard et al., 2009). Serotonin is a vital neurotransmitter that improves environmental adaptability and alleviates oxidative stress (Martin et al., 2000). It is also an important mucosal signalling molecule produced by the enterochromaffin cells in the gut and is related to numerous pathophysiological processes (Coates et al., 2004).

Synthetic amino acids, especially lysine, methionine, threonine and Trp, are regularly supplemented in corn-soybean meal diets. Also, the response of these amino acids on growth performance has been evaluated extensively. However, there is a need to determine the optimum dietary Trp supplemental level which can enhance growth performance with efficient immune response. Therefore, the present study was designed to investigate the optimum level of Trp and its effect on growth performance, immune response and serum parameters in broiler chickens from days 7 to 21.

## 2 | MATERIAL AND METHOD

### 2.1 | Bird management and experimental diets

One hundred and twenty ( $n = 120$ ) 1-day-old broiler chicks were reared in a group and fed a commercial diet for 1 week. At the end of first week, the chicks were divided into three groups in such a way that each group had four replicates with 10 chicks in each replicate. The birds were kept for brooding at 95°F for first week and lowered down by 5°F per week till 85°F was attained. The experimental diets (Table 1) were fed ad libitum. Clean and fresh drinking water was available all time. The experiment lasted for 21 days.

### 2.2 | Performance data

The growth performance in terms of feed intake, BWG and FCR was recorded weekly. On day 21, two chicks per replicate were selected randomly and killed to evaluate carcass characteristics (thigh and breast meat percentage) and visceral organ weight.

### 2.3 | Cellular response

Two birds per replicate were selected and inoculated by avian tuberculin (Veterinary Research Institute Lahore, Pakistan) 0.1 ml and normal saline 0.1 ml between third and fourth interdigital space of right and left foot, respectively, at 19th day of trial. The intra-inflammatory response was measured using screw gauge after 24 and 48 hr of injection and results were interpreted by following the method of Corrier & DeLoach, 1990.

### 2.4 | Humoral response

On day 14, two birds per replicate were administered intravenously with 3% solution of sheep red blood cells to evaluate humoral immune response in terms of overall antibody response, IgM and IgG. After 7 days of injection, blood samples were collected in test tubes and centrifuged at 402 g for 20 min to collect the serum and were freezed at -20°C till further analysis (Delhanty & Solomon, 1966).

### 2.5 | Oxidant and anti-oxidant

The blood samples were collected in EDTA tubes from the slaughtered birds and centrifuged at 2,000 rpm (402g) for 20 min to separate the plasma. The blood plasma was freezed at -20°C for further analysis for the estimation of oxidant and anti-oxidant status. The oxidant status (Erel, 2005), anti-oxidant capacity (Erel, 2004), catalase activity

**TABLE 1** Inclusion level of ingredients in percent

	Control	Trp 0.3%	Trp 0.5%
Ingredients			
Maize	63.5	63.75	63.8
Soybean meal 43%	28	27.75	27.4
Vegetable oil	1.8	1.8	1.8
Lime stone	1.5	1.5	1.5
DCP	1.66	1.66	1.66
Molasses	1.5	1.5	1.5
L-Lysine 55%	0.71	0.71	0.71
DL-methionine	0.22	0.22	0.22
L-threonine	0.21	0.21	0.21
L-tryptophan	<b>0</b>	<b>0.1</b>	<b>0.3</b>
Premix*	0.5	0.4	0.2
NaCl	0.4	0.4	0.4
Total	100	100	100
Nutrient composition (calculated)			
Crude protein	18.39	18.38	18.43
ME Kcal/kg	3,016	3,015	3,011
Fat	4.46	4.46	4.46
Calcium	0.98	0.98	0.98
Av. phosphorus	0.43	0.43	0.43
Sodium	0.20	0.20	0.20
Potassium	0.45	0.45	0.45
Av. Lysine 55%	1.21	1.21	1.20
DL-methionine	0.47	0.47	0.47
L-threonine	0.82	0.82	0.81
Met + Cys	0.82	0.82	0.81
L-tryptophan	<b>0.20</b>	<b>0.3</b>	<b>0.5</b>
Arginine	1.12	1.11	1.10
Isoleucine	0.75	0.74	0.73
Valine	0.78	0.77	0.76

\*Vitamin and mineral both premix were supplemented at 50 g/100 kg of feed. Each Kg of vitamins premix supplied: vitamin A 20,000 KIU; vitamin D<sub>3</sub> 5,400 KIU; vitamin E 48,000 mg; vitamin K<sub>3</sub> 4,000 mg; vitamin B<sub>1</sub> 4,000 mg; vitamin B<sub>2</sub> 9,000 mg; vitamin B<sub>6</sub> 7,600 mg; vitamin B<sub>12</sub> 20 mg; niacin 60,000 mg; pantothenic acid 20,000 mg; folic acid 1,600 mg; biotin 200 mg. Each Kg of minerals premix supplied: iron 10,000 mg; zinc 1,20,000 mg; manganese 1,40,000 mg; copper 12,000 mg; iodine 1,800 mg; cobalt 400 mg; selenium 360 mg. The bold values in the table demonstrate achieved level of tryptophan in the feed.

(Goth, 1991), glutathione reductase (Klotzsch & Bergmeyer, 1965), glutathione peroxidase (Alam, Bristi, & Rafiqzaman, 2013) and aryl-esterase (ARE) activity (Juretic et al., 2006) were analysed.

## 2.6 | Statistical analysis

The data were analysed statistically using GLM procedures of Minitab (17). Significant means were compared by Tukey's test (Steel, Torrie, & Dickey, 1997).

## 3 | RESULTS

### 3.1 | Growth performance from days 7 to 21

The results demonstrated that Trp has no effect on feed intake. The Trp 0.3 and Trp 0.5 tended to increase ( $p < .05$ ) the BWG and improve FCR as compared to control group that was supplemented with 0.2% Trp (Table 2).

### 3.2 | Carcass characteristics

Carcass characteristics (thigh and breast meat), visceral organ weights (liver, kidney, spleen, heart and intestine) remained unchanged by the dietary treatment (Table 3).

### 3.3 | Oxidant and anti-oxidant status

Oxidant and anti-oxidant status of the chicks tended to increase ( $p < .001$ ) as compared to control group while no difference was noted between Trp 0.3 and Trp 0.5 groups. The total oxidant status was lowered ( $p < .05$ ) at 0.5% level of Trp supplementation. Likewise, catalase activity improved ( $p < .05$ ) in chicks fed on Trp 0.5 diet. In addition, glutathione reductase and glutathione peroxidase level improved ( $p < .001$ ) along with ARE activity ( $p < .001$ ) chicks fed diets Trp 0.50 as compared to control group (Table 4).

### 3.4 | Immune response

The data demonstrated that at 24 hr of avian tuberculin inoculation, Trp supplementation increased ( $p < .01$ ) the inflammatory response. However, inflammatory response disappeared after 48 hr of injection (Table 5). The results of humoral immune response showed that the overall antibody titre and IgG increased ( $p < .01$ ) with increasing Trp in diet. But IgM titre was not different among the dietary treatments (Table 6).

## 4 | DISCUSSION

In this study, feed intake remained unchanged by the dietary treatments but BWG and FCR were improved. Similarly, no effect was noticed on carcass traits. These findings are supported by the previous studies (Duarte et al. 2013; Mr & Azam, 2014) in which Trp supplementation had similar response. The similar result for feed intake (Rosebrough, 1996) reported that the feed intake was decreased when broiler chicken was fed by the diet containing low crude protein and excess level of Trp supplement. Duarte et al. (2013) also concluded that Trp had no effect on feed intake in broiler chicken. This result might be due to the crude protein (18.4%) in diet. As Trp is a chief molecule to control behaviour and physiological functions and ultimately required more than national research council (NRC) recommendation for maximum weight gain and FCR in broiler (Cortamira, Seve, Lebreton, & Ganier, 1991; Dong & Zou, 2017; Rosa & Pesti, 2001). The Trp improved BWG and FCR because it is diligently linked to the maintenance of insulin secretion from

**TABLE 2** Effect of tryptophan supplementation on growth performance

Items <sup>†</sup>	Treatment*			p Value
	Control	Trp 0.3	Trp 0.5	
Feed Intake	1,029.9 ± 34.2	1,058.6 ± 25.7	1,006.9 ± 30.4	.507
BWG	616.18 ± 7.11 <sup>b</sup>	704.55 ± 4.88 <sup>a</sup>	728.5 ± 25.5 <sup>a</sup>	.002
FCR	1.67 ± 0.054 <sup>a</sup>	1.50 ± 0.038 <sup>b</sup>	1.38 ± 0.02 <sup>b</sup>	.003

\*Control, Trp 0.3 and Trp 0.5 groups supplemented at 0.2%, 0.3% and 0.5% level of tryptophan respectively. Means that do not share similar letter in row are significantly ( $p < .05$ ) different.

<sup>†</sup>BWG, body weight gain; FCR, Feed conversion ratio. Means that do not share similar letter in row are significantly ( $p < .05$ ) different.

**TABLE 3** Effect of tryptophan supplementation on carcass and visceral organ weight

Items	Treatment*			p Value
	Control	Trp 0.3	Trp 0.5	
Carcass	52.52 ± 6.27	45.12 ± 1.44	42.38 ± 2.35	.232
Spleen	0.14 ± 0.017	0.12 ± 0.004	0.11 ± 0.006	.226
Breast	24.64 ± 2.94	21.16 ± 0.67	19.88 ± 1.1	.226
Thigh	23.46 ± 2.8	20.15 ± 0.64	18.93 ± 1.05	.226
Intestine	9 ± 1.07	7.732 ± 0.24	7.26 ± 0.40	.226
Heart	0.73 ± 0.08	0.63 ± 0.02	0.59 ± 0.03	.226

\*Control, Trp 0.3 and Trp 0.5 groups supplemented at 0.2%, 0.3% and 0.55% level of tryptophan respectively. Means that do not share similar letter in row are significantly ( $p < .05$ ) different.

**TABLE 4** Effect of tryptophan supplementation on oxidant and anti-oxidant status of broiler chicken

Item <sup>†</sup>	Treatments*			p value
	Control	Trp 0.3	Trp 0.5	
TAS mmol/L	1.86 ± 0.22 <sup>b</sup>	4.05 ± 0.27 <sup>a</sup>	3.97 ± 0.20 <sup>a</sup>	.000
TOS μmol/L	21.89 ± 0.34 <sup>a</sup>	16.21 ± 0.79 <sup>ab</sup>	13.6 ± 3.24 <sup>b</sup>	.038
Catalase KU/L	244 ± 11.5 <sup>b</sup>	341.3 ± 13.5 <sup>ab</sup>	388.4 ± 39.4 <sup>a</sup>	.008
GPx μ/mg	2.54 ± 0.468 <sup>c</sup>	22.09 ± 0.91 <sup>b</sup>	27.20 ± 0.99 <sup>a</sup>	.000
GSH μmol/ml	10.20 ± 0.165 <sup>c</sup>	31.72 ± 3.41 <sup>b</sup>	64.63 ± 4.3 <sup>a</sup>	.000
Arylesterase U/L	1958.2 ± 1.83 <sup>b</sup>	1965.9 ± 0.53 <sup>a</sup>	1969.6 ± 0.28 <sup>a</sup>	.000

\*Control, Trp 0.3 and Trp 0.5 groups supplemented at 0.2%, 0.3% and 0.5% level of tryptophan respectively.

<sup>†</sup>TAS, Total anti-oxidant status; TOS, Total oxidant status; GPx, Glutathione peroxidase; GSH, Glutathione reductase. Means that do not share similar letter in row are significantly ( $p < .05$  and  $p < .01$ ) different.

**TABLE 5** Effect of tryptophan supplementation on cellular immunity

Foot pad index	Treatments*			p value
	Control	Trp 0.3	Trp 0.5	
24 hr	0.27 ± 0.032 <sup>c</sup>	0.50 ± 0.030 <sup>b</sup>	0.63 ± 0.031 <sup>a</sup>	.000
48 hr	0.18 ± 0.023	0.21 ± 0.031	0.25 ± 0.040	.430

\*Control, Trp 0.3 and Trp 0.5 groups supplemented at 0.2%, 0.3% and 0.55 level of tryptophan respectively. Means that do not share similar letter in row are significantly ( $p < .01$  and  $p < .05$ ) different.

pancreatic  $\beta$ -cells in growing animals (Clugston & Garlick, 1982; Kim et al., 2014). Thus, insulin is a growth hormone which increases the BWG by increasing the nutrient metabolism. Stress has strong correlation with production performance in broiler. Tryptophan is major precursor of serotonin and had a significant role in the modulation of biological function and diminished stress (Bai et al., 2017). The

catalase, glutathione reductase, glutathione peroxidase and ARE are the main parameters used to evaluate the anti-oxidative status in the enzymatic system, while total anti-oxidant status and total oxidant status represent enzymatic and non-enzymatic systems for oxidative stress. The present study has revealed that Trp has the potential to reduce the oxidative stress and boost up the anti-oxidants in

Items <sup>†</sup>	Treatments*			p value
	Control	Trp 0.3	Trp 0.5	
Total Antibodies	1.68 ± 0.073 <sup>c</sup>	1.98 ± 0.073 <sup>b</sup>	2.52 ± 0.073 <sup>a</sup>	.000
IgG	1.14 ± 0.060 <sup>b</sup>	1.32 ± 0.073 <sup>b</sup>	1.98 ± 0.073 <sup>a</sup>	.000
IgM	0.54 ± 0.060	0.66 ± 0.060	0.54 ± 0.060	.3

\*Control, Trp 0.3 and Trp 0.5 groups supplemented at 0.2%, 0.3% and 0.55 level of tryptophan respectively.

<sup>†</sup>Total Antibodies, antibodies against sheep RBCs; IgG, Immunoglobulin G; IgM, Immunoglobulin M. Means that do not share similar letter in rows are significantly ( $p < .01$  and  $p < .05$ ) different.

the broiler birds. Improved anti-oxidant status mimics the stress in broiler chicken and ultimately facilitate to optimum weight gain and FCR. The similar results were coded by other investigations in which Liu et al. (2015) reported that in White Pekin ducks the anti-oxidant, in terms of glutathione peroxidase (GSH-Px) and catalase (CAT), has been increased meaningfully not only in serum and liver but also in breast muscles by dietary Trp supplementation above the NRC (1994) recommendation. Patil et al. (2013) demonstrated that the L-tryptophan is the precursor of serotonin and melatonin and inhibited the oxidative damage in broiler birds, improved enzymatic action of catalase and superoxide dismutase. Wang, Min, Yuan, Zhang, & Guo (2014) study revealed that 1.5-fold increase in dietary supplementation of Trp level than recommended could lessen oxidative stress and improve feed efficiency in broilers under stress condition. The ARE activity as an anti-oxidant improved by Trp and its activity is associated with the paraoxonase-1 (PON1) gene that is identified to defend lipoproteins from oxidation and offer defence in contrast to metabolic syndrome (De la Iglesia, Mansego, Sanchez-Muniz, Zulet, & Martinez, 2014). Therefore, adding Trp above NRC (1994) can maximize the concentration of anti-oxidants in the serum and may protect cells from oxidative injury by clearing ROS.

The present study showed that the Trp increased the cellular and humoral immunity in broiler chicken. Sanchez, Sanchez, Paredes, Rodriguez, and Barriga (2008) suggested that the Trp supplementation stimulated melatonin synthesis and augmented the innate immune response carried out by peritoneal macrophages as a consequence of the immune-regulatory action of melatonin. The effect of Trp on immune function may be directed via melatonin receptors located on immune tissues. Similar results were illustrated by Dong et al. (2012) the immune response by Trp supplementation in Babcock Brown layers during summer climate. The study concluded that Trp supplementation was effective in the regulation of IgM, quadratically. Immune response against IBDV has been evaluated in broiler by supplementing Trp and it was concluded that Trp supplementation improved the immunoglobulin-G level to combat against diseases (Emadi et al., 2011; Mozhdeh et al., 2010). Gershoff, Gill, Simonian, and Steinberg (1968) observed that a deficiency of Trp decreased the antibody production. Esteban et al. (2004) indicated that the synthesis of serotonin and melatonin, as well as the innate immune response, can be modulated by Trp.

In conclusion, Trp is third or fourth limiting amino acid in poultry diets. The study proposed that dietary Trp supplementation

level should be greater than NRC (1996) recommendations. Tryptophan level of 0.3% and 0.5% as compared to 0.2% improved growth performance but had no effect on carcass characteristics. Tryptophan level above the NRC recommendation improved the anti-oxidant status, humoral and cellular immunity in broiler chicken during 7–21 days.

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#### CONFLICT OF INTEREST

There is no conflict of interest.

#### ETHICAL STATEMENT

All the experimental proceedings in this experiment were approved by the university animal ethics committee.

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#### REFERENCES

- Alam, M. N., Bristi, N. J., & Rafiqzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21, 143–152. <https://doi.org/10.1016/j.jsps.2012.05.002>
- Bai, M., Liu, H., Xu, K., Oso, A. O., Wu, X., Liu, G., ... Yin, Y. (2017). A review of the immunomodulatory role of dietary tryptophan in livestock and poultry. *Amino Acids*, 49, 67–74. <https://doi.org/10.1007/s00726-016-2351-8>
- Clugston, G., & Garlick, P. (1982). The response of protein and energy metabolism to food intake in lean and obese man. *Human Nutrition. Clinical Nutrition*, 36, 57–70.
- Coates, M. D., Mahoney, C. R., Linden, D. R., Sampson, J. E., Chen, J., Blaszyk, H., ... Mawe, G. M. (2004). Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome 1. *Gastroenterology*, 126, 1657–1664. <https://doi.org/10.1053/j.gastro.2004.03.013>
- Corrier, D., & DeLoach, J. (1990). Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poultry Science*, 69, 403–408. <https://doi.org/10.3382/ps.0690403>

- Cortamira, N., Seve, B., Lebreton, Y., & Ganier, P. (1991). Effect of dietary tryptophan on muscle, liver and whole-body protein synthesis in weaned piglets: Relationship to plasma insulin. *British Journal of Nutrition*, 66, 423–435. <https://doi.org/10.1079/BJN19910045>
- De la Iglesia, R., Mansego, M. L., Sanchez-Muniz, F. J., Zulet, M. A., & Martinez, J. A. (2014). Arylesterase activity is associated with antioxidant intake and paraoxonase-1 (pon1) gene methylation in metabolic syndrome patients following an energy restricted diet. *EXCLI Journal*, 13, 416.
- Delhanty, J., & Solomon, J. (1966). The nature of antibodies to goat erythrocytes in the developing chicken. *Immunology*, 11, 103.
- Dong, X., Azzam, M., Rao, W., Yu, D., & Zou, X. (2012). Evaluating the impact of excess dietary tryptophan on laying performance and immune function of laying hens reared under hot and humid summer conditions. *British Poultry Science*, 53, 491–496. <https://doi.org/10.1080/00071668.2012.719149>
- Dong, X., & Zou, X. (2017). *Effects of excess dietary tryptophan on laying performance, antioxidant capacity and immune function of laying hens, amino acid-new insights and roles in plant and animal*. InTech.
- Duarte, K. F., Junqueira, O. M., Filardi, R. D. S., Siqueira, J. C. D., Puzotti, M. M., Garcia, E. A., ... Laurentiz, A. C. D. (2013). Digestible tryptophan requirements for broilers from 22 to 42 days old. *Revista Brasileira De Zootecnia*, 42, 728–733. <https://doi.org/10.1590/S1516-35982013001000006>
- Emadi, M., Jahanshiri, F., Kaveh, K., Hair-Bejo, M., Ideris, A., & Alimon, A. (2011). Nutrition and immunity: The effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. *Avian Pathology*, 40, 63–72. <https://doi.org/10.1080/03079457.2010.539590>
- Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, 37, 277–285. <https://doi.org/10.1016/j.clinbiochem.2003.11.015>
- Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38, 1103–1111. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>
- Esteban, S., Nicolaus, C., Garmundi, A., Rial, R. V., Rodriguez, A. B., Ortega, E., & Ibars, C. B. (2004). Effect of orally administered L-tryptophan on serotonin, melatonin, and the innate immune response in the rat. *Molecular and Cellular Biochemistry*, 267, 39–46. <https://doi.org/10.1023/B:MCBI.0000049363.97713.74>
- Gershoff, S. N., Gill, T. J. III, Simonian, S. J., & Steinberg, A. I. (1968). Some effects of amino acid deficiencies on antibody formation in the rat. *The Journal of Nutrition*, 95, 184–190. <https://doi.org/10.1093/jn/95.2.184>
- Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196, 143–151. [https://doi.org/10.1016/0009-8981\(91\)90067-M](https://doi.org/10.1016/0009-8981(91)90067-M)
- Halliwell, B., & Gutteridge, J. (1999). Mechanism of damage of cellular targets by oxidative stress: Lipid peroxidation. *Free Radicals in Biology and Medicine*, 284, 313.
- Juretic, D., Motejlkova, A., Kunovic, B., Rekić, B., Flegar-Mestric, Z., Vujic, L., ... Simeon-Rudolf, V. (2006). Paraoxonase/arylesterase in serum of patients with type II diabetes mellitus. *Acta Pharmaceutica*, 56, 59–68.
- Kim, K., Oh, C.-M., Ohara-Imaizumi, M., Park, S., Namkung, J., Yadav, V. K., ... Kim, H. (2014). Functional role of serotonin in insulin secretion in a diet-induced insulin-resistant state. *Endocrinology*, 156, 444–452. <https://doi.org/10.1210/en.2014-1687>
- Klotzsch, H., & Bergmeyer, H. U. (1965). *Glutathione, methods of enzymatic analysis* (363–366). Elsevier.
- Li, P., Yin, Y. L., Li, D., Kim, S. W., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, 98, 237–252. <https://doi.org/10.1017/S000711450769936X>
- Liu, Y., Yuan, J., Zhang, L., Zhang, Y., Cai, S., Yu, J., & Xia, Z. (2015). Effects of tryptophan supplementation on growth performance, antioxidative activity, and meat quality of ducks under high stocking density. *Poultry Science*, 94, 1894–1901. <https://doi.org/10.3382/ps/pev155>
- Martin, C., Duclos, M., Aguerre, S., Mormede, P., Manier, G., & Chaouloff, F. (2000). Corticotropic and serotonergic responses to acute stress with/without prior exercise training in different rat strains. *Acta Physiologica Scandinavica*, 168, 421–430. <https://doi.org/10.1046/j.1365-201x.2000.00683.x>
- Mozhdeh, E., Fatemeh, J., Kamran, K., Mohd, H. B., Aini, I., & Razak, A. (2010). Tryptophan stimulates immune response in broiler chickens challenged with infectious bursal disease vaccine. *Journal of Animal and Veterinary Advances*, 9, 610–616.
- Mr, E. G., & Azzam, M. (2014). Effects of dietary tryptophan levels and stocking density during the growing-finishing phase on broiler performance and immunity. *Asian Journal of Animal and Veterinary Advances*, 9, 568–577.
- Patil, R., Tyagi, J., Sirajudeen, M., Singh, R., Moudgal, R., & Mohan, J. (2013). Effect of dietary melatonin and L-tryptophan on growth performance and immune responses of broiler chicken under experimental aflatoxicosis. *Iranian Journal of Applied. Animal Science*, 3, 139–144.
- Richard, D. M., Dawes, M. A., Mathias, C. W., Acheson, A., Hill-Kapturczak, N., & Dougherty, D. M. (2009). L-tryptophan: Basic metabolic functions, behavioral research and therapeutic indications. *International Journal of Tryptophan Research*, 2, S2129.
- Rosa, A., & Pesti, G. (2001). Estimation of the tryptophan requirement of chickens for maximum body weight gain and feed efficiency. *Journal of Applied Poultry Research*, 10, 135–140. <https://doi.org/10.1093/japr/10.2.135>
- Rosebrough, R. (1996). Crude protein and supplemental dietary tryptophan effects on growth and tissue neurotransmitter levels in the broiler chicken. *British Journal of Nutrition*, 76, 87–96. <https://doi.org/10.1079/BJN19960011>
- Sanchez, S., Sanchez, C. L., Paredes, S. D., Rodriguez, A. B., & Barriga, C. (2008). The effect of tryptophan administration on the circadian rhythms of melatonin in plasma and the pineal gland of rats. *Journal of Applied Biomedicine*, 6. <https://doi.org/10.32725/jab.2008.021>
- Steel, R. G., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: A biological approach*. McGraw-Hill.
- Surai, P. F. (2015). Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. *Antioxidants*, 4, 204–247. <https://doi.org/10.3390/antiox4010204>
- Wang, B., Min, Z., Yuan, J., Zhang, B., & Guo, Y. (2014). Effects of dietary tryptophan and stocking density on the performance, meat quality, and metabolic status of broilers. *Journal of Animal Science and Biotechnology*, 5, 44.
- Yue, Y., Guo, Y., & Yang, Y. (2017). Effects of dietary L-tryptophan supplementation on intestinal response to chronic unpredictable stress in broilers. *Amino Acids*, 49, 1227–1236. <https://doi.org/10.1007/s00726-017-2424-3>

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