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.
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 administrated 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
MUND et al. (Goth, 1991), glutathione reductase (Klotzsch & Bergmeyer, 1965), glutathione peroxidase (Alam, Bristi, & Rafiquzzaman, 2013) and ary-lesterase (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) 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 1 Inclusion level of ingredients in percent

| Ingredients   | Control | Trp 0.3% | Trp 0.5% |
|---------------|---------|---------|---------|
| Maize         | 63.5    | 63.75   | 63.8    |
| Soybean meal  | 43%     | 28      | 27.75   |
| 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  | 0       | 0.1     | 0.3     |
| Premix*       | 0.5     | 0.4     | 0.2     |
| NaCl          | 0.4     | 0.4     | 0.4     |
| Total         | 100     | 100     | 100     |

| Nutrient composition (calculated) | Control | Trp 0.3% | Trp 0.5% |
|----------------------------------|---------|---------|---------|
| 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                     | 0.20    | 0.3     | 0.5     |
| 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 Kgf of vitamins premix supplied: vitamin A 20,000 KIU; vitamin D₃ 5,400 KIU; vitamin E 48,000 mg; vitamin K₃ 4,000 mg; vitamin B₁ 4,000 mg; vitamin B₂ 9,000 mg; vitamin B₆ 7,600 mg; vitamin B₁₂ 20 mg; niacin 60,000 mg; pantothenic acid 20,000 mg; folic acid 1,600 mg; biotin 200 mg. Each Kgf 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 demostrate achieved level of tryptophan in the feed.
pancreatic β-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

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

| Items       | 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\*b | 704.55 ± 4.88\*a | 728.5 ± 25.5\*a | .002 |
| FCR         | 1.67 ± 0.054\*a    | 1.50 ± 0.038\*b | 1.38 ± 0.02\*b | .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.

†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 |
| Breast      | 23.46 ± 2.8  | 20.15 ± 0.64 | 18.93 ± 1.05 | .226 |
| Intestine   | 9 ± 1.07 | 7.73 ± 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 0.5% 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†        | Treatment*           | p value |
|--------------|----------------------|---------|
| Control      | Trp 0.3 | Trp 0.5 |         |
| TAS mmol/L   | 1.86 ± 0.22\*b | 4.05 ± 0.27\*a | 3.97 ± 0.20\*a | .000 |
| TOS µmol/L   | 21.89 ± 0.34\*a | 16.21 ± 0.79\*ab | 13.6 ± 3.24\*b | .038 |
| Catalase KU/L| 244 ± 11.5\*b     | 341.3 ± 13.5\*ab | 388.4 ± 39.4\*a | .008 |
| GPx μ/µg     | 2.54 ± 0.468\*c   | 22.09 ± 0.91\*b | 27.20 ± 0.99\*a | .000 |
| GSH µmol/ml  | 10.20 ± 0.165\*c  | 31.72 ± 3.41\*b | 64.63 ± 4.3\*a | .000 |
| Arylesterase U/L | 1958.2 ± 1.83\*b | 1965.9 ± 0.53\*a | 1969.6 ± 0.28\*a | .000 |

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

†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 | Treatment*           | p value |
|---------------|----------------------|---------|
| Control       | Trp 0.3 | Trp 0.5 |         |
| 24 hr         | 0.27 ± 0.032\*c | 0.50 ± 0.030\*b | 0.63 ± 0.031\*a | .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.
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, 2010). Gershoff, Gill, Simonian, and Steinberg (1968) observed that 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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**TABLE 6 Effect of Tryptophan supplementation on humoral immunity**

| Items† | Treatments* | Control | Trp 0.3 | Trp 0.5 | p value |
|--------|-------------|---------|---------|---------|---------|
| Total Antibodies | 1.68 ± 0.073a | 1.98 ± 0.073b | 2.52 ± 0.073a | .000 |
| IgG    | 1.14 ± 0.060b | 1.32 ± 0.073b | 1.98 ± 0.073a | .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.
†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.
MUND et al.

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