Evaluation of tryptophan biomass as an alternative to conventional crystalline tryptophan in broiler diets

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Primary Audience: Nutritionists, Broiler Producers

SUMMARY

The current experiment was conducted to evaluate tryptophan (**Trp**) biomass as an alternative to conventional crystalline Trp in broiler diets. In a growth trial, a total of 288 eight-day-old chicks were randomly allotted to 1 of 3 treatments with 8 replicates. A negative control (**NC**) diet was formulated to contain 40% less amounts of digestible Trp than its requirement. A positive control (**PC**) diet was formulated to meet digestible Trp requirement by supplementing with crystalline Trp. An additional diet was prepared by supplementing Trp biomass to have equal amounts of digestible Trp in the PC diet. In a metabolism trial, a total of 54 forty-two-day-old chickens were allotted 1 of 3 dietary treatments used in the growth trial with 9 replicates to evaluate the effect of dietary Trp biomass on gross energy (**GE**) and nitrogen (**N**) utilization in broiler diets. Results indicated that PC and Trp biomass treatments had greater ($P < 0.05$) body weight gain (**BWG**) and feed intake (**FI**) than NC treatment. The Trp biomass treatment showed no differences in BW, BWG, and FI compared with PC treatment. The PC treatment had less ($P < 0.05$) blood heterophil to lymphocyte ratio than NC treatment. The PC and Trp biomass treatments had greater ($P < 0.05$) villus height to crypt depth ratio than NC treatment. Inclusion of Trp biomass did not affect GE and N utilization in broiler diets. In conclusion, Trp biomass can be used as a potential alternative to conventional crystalline Trp in broiler diets.

Key words: broiler chicken, crystalline tryptophan, growth performance, intestinal health, tryptophan biomass

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DESCRIPTION OF PROBLEM

Tryptophan (**Trp**) is an essential amino acid (**AA**) in animals. Its primary roles include serving as a structural component of proteins and a precursor of niacin, and therefore, an adequate supply of Trp in diets is essential to maintain optimal physiological functions such as growth, nutrient metabolism, and tissue synthesis in animals (Corzo et al., 2005). Furthermore, Trp is a precursor of serotonin and melatonin, which induce specific physiological and behavioral changes in animals. Serotonin is a vital neurotransmitter that can improve adaptability to environmental changes, modify intestinal activity, and alleviate oxidative stress (Martin et al., 2000; Wensley et al., 2020). Melatonin regulates reproduction (Wang et al., 2014) and immunity (Zhou et al., 2016), and exhibits anticancer (Söderquist et al., 2016), antiaging (Tamura et al., 2017), and antioxidative

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actions (Reiter, 1993; Zhang et al., 2006; Mehaisen et al., 2015). Therefore, additional supply of dietary Trp has recently gained increasing attention in the feed industry.

Crystalline Trp is widely used for supplemental Trp in feeds. This crystalline Trp is produced by the fermentation of bacteria including mutant strains of *Corynebacterium glutamicum* with carbohydrates and other nutrients in a culture medium (Leuchtenberger et al., 2005). After several fermentation steps, crystalline Trp is obtained as a final product from the purification of the fermented biomass (Hermann, 2003; Wensley et al., 2020). However, this purification process has some possible disadvantages such as inevitable Trp loss, increasing production costs, and increasing disposal of biomass wastes. Therefore, the use of the raw Trp biomass without purification steps in animal diets may provide economic benefits. Moreover, the unpurified Trp biomass may contain high amounts of *Corynebacterium glutamicum* cell mass that was reported to improve animal performance and intestinal health (Cheng et al., 2021). In addition, Trp biomass may also contain some postbiotics such as AA, phosphorus, and functional compounds in addition to Trp (Almeida et al., 2014; Wensley et al., 2020). Consequently, it is expected that Trp biomass can be a potential alternative to crystalline Trp, and further may provide possible benefits on animal health and production. Several previous studies have evaluated the use of various AA biomass such as Lys biomass, Val biomass, and Thr biomass in swine and poultry diets, which reported that the AA biomass can be a potential alternative to conventional feed-grade crystalline AA (Sulabo et al., 2013; Wensley et al., 2020; Jespersen et al., 2021). However, the information regarding the use of Trp biomass as an alternative to crystalline Trp, especially in poultry diets, is still lacking.

Therefore, the current experiment aimed to evaluate Trp biomass as an alternative to conventional crystalline Trp in broiler diets.

MATERIALS AND METHODS

All experimental procedures in the current study were reviewed and approved by the Animal Care and Use Committee at Chung-Ang University (approval number: 2021-00051).

Animals, Experimental Design, and Diets

In a growth trial (Experiment 1), a total of 500 one-day-old Ross 308 broiler chicks were obtained from a local hatchery (Dongsan hatchery, Cheonan, Republic of Korea). When birds were arrived at the research facility, all birds were divided by the sex and raised separately in the male or female cages. All chicks were fed a crumble-formed commercial starter diet until 7 d of age. On 8 d of age, all chicks were weighed and 288 chicks with a narrow BW of 206 ± 0.2 g were finally selected to obtain the homogeneous BW at the start of the experiment. The selected chicks were randomly allotted to 1 of 3 treatments with 8 replicates consisting of 6 male and 6 female birds per replicate. All birds were housed in battery cages (76 × 78 × 45 cm, width × length × height). The Trp biomass (TRP pro, CJ Bio, Seoul, Republic of Korea) with a pale and dark brown free-flowing appearance was used in this experiment (Table 1). The Trp biomass was analyzed for DM (method 930.15; AOAC, 2005) and CP (method 990.03; AOAC, 2005). The concentrations of gross energy (**GE**) in Trp biomass were analyzed using bomb calorimetry (Model 6400, Parr instruments Co., Moline, IL). The AA

Table 1. Analyzed nutrient composition of Trp biomass used in this experiment (%, as-fed basis).

Items	Value
DM (%)	96.64
CP (%)	75.04
Gross energy (kcal/kg)	6,089
Total amino acid (%)	
Trp	61.49
Arg	1.17
His	0.40
Ile	0.77
Leu	1.49
Lys	0.90
Met	0.37
Phe	0.86
Thr	1.08
Val	1.06
Ala	1.83
Asp	2.12
Cys	0.20
Glu	2.94
Gly	1.00
Pro	0.57
Ser	0.92
Tyr	0.72

concentrations in Trp biomass were analyzed (Ultimate 3000, Thermo Dionex, Sunnyvale, CA) and used for diet formulation. A 2-phase feeding program with a grower diet from 8 to 21 d (Table 2) and a finisher diet from 22 to 34 d of age (Table 3) was adopted for the 27-d feeding trial. A negative control (**NC**) diet was prepared mainly with corn, corn gluten meal, distillers dried grains with solubles (**DDGS**), and canola meal to produce a Trp-deficient diet. Therefore, the NC diet contained 40% less amounts of digestible Trp (0.108% and 0.096% digestible Trp in grower and finisher phases, respectively) than digestible Trp requirement for each phase (Aviagen, 2019). A positive control (**PC**) diet was formulated to meet digestible Trp requirement by the inclusion of crystalline Trp (Aviagen, 2019). The Trp biomass treatment diet was prepared by inclusion of Trp biomass to produce equal amounts of digestible Trp in the PC diet. The pre-determined concentration of digestible Trp in Trp biomass was 60.26%, which was used to formulate diets. All nutrient concentrations, except for digestible Trp, were equalized among dietary treatments in accordance with the recommended nutrient concentrations for each phase of broiler chickens (Aviagen, 2019).

Experimental diets and water were provided on an ad libitum basis for 27 d. The room temperature was maintained at 28˚C at the start of the experiment and then gradually decreased to 24˚C as recommended by the Ross 308 manual (Aviagen, 2019). A 23-h lighting schedule was applied during the experimental period. The BW, body weight gain (**BWG**), and feed intake (**FI**) were measured at 21 d of age and the end of the experiment. The mortality was recorded daily. The FCR was calculated by dividing FI by BWG after correcting for mortality.

In a metabolism trial (Experiment 2), the effects of inclusion of Trp biomass on GE and nitrogen (**N**) utilization in broiler diets were evaluated. A total of 54 forty-two-day-old chickens were selected based on the final BW at the end of the growth trial (Experiment 1) and had similar BW at the start of the metabolism trial. All birds were allotted 1 of 3 dietary treatments with 9 replicates consisting of 2 birds (1 male and 1 female) per replicate. The selected brids were assinged to the same treatment diet as they were fed during the finishing period of the growth trial. Birds were placed in

Table 2. Composition and nutrient concentration of the experimental diets from 8 to 21 d of age.

	Dietary treatments		
Items	NC	PC	Trp biomass
Ingredients (%)			
Corn grains	58.49	58.64	58.72
Corn gluten meal	14.31	14.30	14.28
DDGS[1]	10.00	10.00	10.00
Canola meal	8.04	8.04	8.04
Soybean oil	0.30	0.22	0.18
MDCP[2]	1.52	1.52	1.52
Limestone	1.46	1.46	1.46
Celite	1.500	1.427	1.380
DL-Met (99%)	0.27	0.27	0.27
Lys-HCl (98%)	0.79	0.79	0.79
Thr (99%)	0.28	0.28	0.28
Ala (99%)	2.01	1.95	1.93
L-Trp (98%)	0.000	0.073	0.000
Trp biomass[3]	0.000	0.000	0.120
NaCl	0.20	0.20	0.20
Choline (50%)	0.18	0.18	0.18
$NaHCO_3$	0.30	0.30	0.30
Coccidiostats	0.10	0.10	0.10
Antioxidant	0.05	0.05	0.05
Vitamin premix[4]	0.10	0.10	0.10
Mineral premix[5]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated energy and nutrients[6]			
AME_n (kcal/kg)	3,120	3,120	3,120
CP (%)	21.50	21.50	21.50
Digestible Lys (%)	1.15	1.15	1.15
Digestible Met+Cys (%)	0.87	0.87	0.87
Digestible Met (%)	0.59	0.59	0.59
Digestible Thr (%)	0.77	0.77	0.77
Digestible Trp (%)	0.108	0.180	0.180
Total calcium (%)	0.87	0.87	0.87
Available phosphorus (%)	0.44	0.44	0.44

[1]DDGS, distillers dried grains with solubles.

[2]MDCP, monodicalcium phosphate.

[3]Trp biomass contained 61.49% total Trp and 60.26% digestible Trp.

[4]Provided per kg of the complete diet: vitamin A, 12,000 IU (retinyl acetate); vitamin D_3, 4,000 IU; vitamin K_3, 4.0 mg (menadione dimethylpyrimidinol); vitamin B_1, 4.0 mg; vitamin B_2, 10.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20.0 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg.

[5]Provided per kg of the complete diet: iron, 60 mg ($FeSO_4$); zinc, 100 mg ($ZnSO_4$); manganese, 120 mg (MnO); copper, 16 mg ($CuSO_4$); cobalt, 1,000 μg ($CoSO_4$); selenium, 300 μg (Na_2SeO_3); iodine, 1.25 mg [$Ca(IO_3)_2$].

[6]Calculated values from Ross 308 broiler nutrition specifications (Aviagen, 2019).

Table 3. Composition and nutrient concentration of the experimental diets from 22 to 34 d of age.

	Dietary treatments		
Items	NC	PC	Trp biomass
Ingredients (%)			
Corn grains	65.27	65.47	65.60
Corn gluten meal	16.93	16.81	16.72
DDGS[1]	8.00	8.00	8.00
Canola meal	2.39	2.39	2.39
Soybean oil	0.16	0.08	0.05
MDCP[2]	1.46	1.46	1.43
Limestone	1.38	1.38	1.38
Celite	1.500	1.435	1.393
DL-Met (99%)	0.22	0.22	0.22
Lys-HCl (98%)	0.76	0.76	0.76
Thr (99%)	0.23	0.23	0.23
Ala (99%)	0.67	0.67	0.69
L-Trp (98%)	0.000	0.065	0.000
Trp biomass[3]	0.000	0.000	0.107
NaCl	0.20	0.20	0.20
Choline (50%)	0.18	0.18	0.18
$NaHCO_3$	0.30	0.30	0.30
Coccidiostats	0.10	0.10	0.10
Antioxidant	0.05	0.05	0.05
Vitamin premix[4]	0.10	0.10	0.10
Mineral premix[5]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated energy and nutrients[6]			
AME_n (kcal/kg)	3,220	3,220	3,220
CP (%)	19.50	19.50	19.50
Digestible Lys (%)	1.06	1.06	1.06
Digestible Met +Cys (%)	0.80	0.80	0.80
Digestible Met (%)	0.54	0.54	0.54
Digestible Thr (%)	0.69	0.69	0.69
Digestible Trp (%)	0.096	0.160	0.160
Total calcium (%)	0.79	0.79	0.79
Available phosphorus (%)	0.40	0.44	0.44

[1]DDGS, distillers dried grains with solubles.

[2]MDCP, monodicalcium phosphate.

[3]Trp biomass contained 61.49% total Trp and 60.26% digestible Trp.

[4]Provided per kg of the complete diet: vitamin A, 12,000 IU (retinyl acetate); vitamin D_3, 4,000 IU; vitamin K_3, 4.0 mg (menadione dimethylpyrimidinol); vitamin B_1, 4.0 mg; vitamin B_2, 10.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20.0 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg.

[5]Provided per kg of the complete diet: iron, 60 mg ($FeSO_4$); zinc, 100 mg ($ZnSO_4$); manganese, 120 mg (MnO); copper, 16 mg ($CuSO_4$); cobalt, 1,000 μg ($CoSO_4$); selenium, 300 μg (Na_2SeO_3); iodine, 1.25 mg [$Ca(IO_3)_2$].

[6]Calculated values from Ross 308 broiler nutrition specifications (Aviagen, 2019).

metabolic cages (35.2 × 45.0 × 55.3 cm, width × length × height). The room temperature was set at 21°C and a 23-h lighting schedule was applied during the experimental period. Three diets (i.e., NC, PC, and Trp biomass) used in the growth trial were evaluated. All birds were provided with feed and water ad libitum before the start of the metabolism trial. The experimental procedure in the metabolism trial was followed by the method of Kim et al. (2021b) with a minor modification. In brief, at the start of the experiment (42 d of age), the average FI was determined using the basal diet for 2 d. Afterward, all birds were subjected to a 72-h adaptation period. For the first 55 h, birds were fed the designated treatment diets at 80% average FI (i.e., 350 g per cage) to minimize ingredient selections in feeders, and birds were then subjected to fasting for 17 h to empty the gastrointestinal tract before the start of the collection period. The collection period lasted for 96 h. Birds were fed 350 g of each treatment diet at 11:00 AM for the first 79 h (i.e., 4 times) and fasted for the next 17 h. Excreta were collected continuously during the whole collection period. The collected excreta were immediately stored at −20°C. The excreta samples were dried in a forced-air drying oven at 60°C for 48 h and finely ground for further analysis. The samples for diets and excreta were analyzed for GE using bomb calorimetry (Model 6400; Parr Instruments Co., Moline, IL) and N (method 990.03; AOAC, 2005). The GE and N retention (%) as well as apparent metabolizable energy (**AME**) and N-corrected AME (**AME_n**) values of treatment diets were calculated based on the previous method (Kim et al., 2021b, 2022).

Sample Collection and Analysis

At the conclusion of the growth trial, 1 male and 1 female broiler chickens, whose BW was the closest to the average BW per replicate, were euthanized by CO_2 asphyxiation. Both male and female birds were used for the analysis of meat quality, immune response, intestinal health status, and expression of genes related to tight junction and inflammatory cytokine in the jejunum mucosa. The breast meat was collected

and stored at 4°C before analysis. From the breast meat, pH, meat colors, water holding capacity (**WHC**), and thiobarbituric acid reactive substances (**TBARS**) were measured based on the previous methods (Goo et al., 2019; Choi et al., 2021). The jejunal fragments were also collected for analyzing intestinal morphology. The jejunal mucosa samples were collected and stored at −80°C before analyzing gene expressions.

The selected male bird was also used for the analysis of blood heterophil to lymphocyte ratio (**H:L ratio**), feather corticosterone (**CORT**), and immune responses. Blood samples were collected via heart puncture into a 10-mL EDTA (Becton and Dickinson Company, Diagnostics, UK). As a stress indicator, blood H:L ratio was analyzed by the method of Lentfer et al. (2015) with a minor modification. The detailed procedure was reported in our previous experiment (Yu et al., 2021). Feather samples were also collected from primary flight feathers and used for the analysis of feather CORT concentrations as another stress indicator (Lee et al., 2022). The CORT concentrations in the feather were analyzed by the method of Bortolotti et al. (2008) with a minor modification. The detailed procedure was reported in our previous experiment (Lee et al., 2022). A cutaneous basophil hypersensitivity (**CBH**) response test was conducted to measure cell-mediated immune responses in accordance with the methods of Kean and Lamont (1994). The detailed procedure was described in the previous experiment (Kim et al., 2021a).

Jejunal Morphology

The jejunal morphology was examined by the method of Nari et al. (2020). In short, 10% buffered formalin was used to flush and fix the sample. The jejunum fragment was embedded in paraffin. A 5-μm section of each paraffin-embedded sample was placed onto a glass slide, stained with hematoxylin-eosin, and examined under a light microscope. The villus height (**VH**), crypt depth (**CD**), villus width (**VW**), and VH to CD ratio (**VH:CD ratio**) were measured and calculated by the method of Wiersema et al. (2021) with a minor modification. There were 20 measurements per jejunal sample. The average value from these 20 measurements was used as the mean of each measurement. The detailed procedure was reported in the previous experiment (He et al., 2020).

Antioxidant Status in the Jejunal Mucosa

A portion of jejunal mucosa samples was collected for the analysis of antioxidant status such as total antioxidant capacity (**TAC**), reactive oxygen species (**ROS**), and malondialdehyde (**MDA**). The antioxidant status in the jejunal mucosa was determined using available kits OxiSelect TAC assay kit (STA-360, Cell Biolabs, San Diego, CA), OxiSelect In Vitro ROS/RNS assay kit (Green Fluorescence, STA-347, Cell Biolabs, San Diego, CA), and OxiSelect thiobarbituric acid reactive substance assay Kit (MDA Quantitation, STA-330, San Diego, CA). Protein concentrations were also analyzed using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL). The detailed procedure of antioxidant status analysis was reported previously (Yu et al., 2021).

Gene Expression in the Jejunal Mucosa

The expression levels of tight junction-related genes, pro-inflammatory cytokine genes, and anti-inflammatory genes in the jejunal mucosa were analyzed by the method of Shin et al. (2018). The primers used in this experiment were designed using NCBI/Primer-BLAST (Table 4). The primers of selected genes for tight-junction (OCLN, CLDN-1, ZO-1, and JAM2), pro-inflammatory cytokines (IFN-γ, IL-1β, and IL-6), and anti-inflammatory cytokines (IL-4, IL-10, and TGF-β_4) were designed based upon sequences available in public databases and synthesized by Genotec Co. Ltd. (Daejeon, Republic of Korea). The specificity of the primers was confirmed by PCR amplification as demonstrated by Aznar and Alarcon (2002). The relative quantification of gene-specific expressions was calculated using the $2^{-\Delta\Delta Ct}$ method after normalization to glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**; Thomsen et al., 2010). These analyses were performed at the BT research facility center, Chung-Ang University.

Table 4. Sequence of the primers used in real-time quantitative PCR.

Target genes[1]	Primer sequence (5'-3')	Tm[4]	Product size (bp)	Accession no.
GAPDH	F[2]: TGCTGCCCAGAACATCATCC R[3]: ACGGCAGGTCAGGTCAACAA	50 - 65	142	NM_204305
OCLN	F[2]: TCGTGCTGTGCATCGCCATC R[3]: CGCTGGTTCACCCCTCCGTA	60	178	NM_205128
CLDN-1	F[2]: CAGACTCTAGGTTTTGCCTT R[3]: AATCTTTCCAGTGGCGATAC	58.3	149	NM_001013611
ZO-1	F[2]: CACACTGTGACCCCAAAA R[3]: AAGGTCCATCTCAGTTTCAC	56.3	151	XM_040680632
JAM-2	F[2]: GGGGGTCTTCTGCTATCAT R[3]: GGGACTGGATTTTCTTCCAT	56.0	155	NM_001397141
IFN-γ	F[2]: AGCTGACGGTGGACCTATTATT R[3]: GGCTTTGCGCTGGATTC	60	259	Y07922
IL-1β	F[2]: GAATTCTTTGACAGTCTGCG R[3]: TCGGGTTGGTTGGTGATG	60	244	Y15006
IL-6	F[2]: TCATCCTCCGAGACTTTACT R[3]: CCGAACTAAAACATTCAGGC	58.3	100	NM_204628
IL-4	F[2]: ACCCAGGGCATCCAGAAG R[3]: CAGTGCCGGCAAGAAGTT	60	258	AJ621735
IL-10	F[2]: CAGGGACGATGAACTTAACA R[3]: AGGACCTCATCAGTGTAGAA	55.2	117	NM_001004414
TGF-β_4	F[2]: CGGGACGGATGAGAAGAAC R[3]: CGGCCCACGTAGTAAATGAT	60	178	NM_205128

[1]Abbreviations: CLDN-1; claudin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN-γ, interferon gamma; IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-4, interleukin 4; IL-10, interleukin 10; JAM-2, junctional adhesion molecule B; OCLN, occludin; TGF-β_4, transforming growth factor beta-4; ZO-1; zonula occludens-1.

[2]F = Forward.

[3]R = Reverse.

[4]Tm, amplification temperature,°C.

Statistical Analysis

All data were analyzed by one-way ANOVA using PROC GLM procedure of SAS (Version 9.4; SAS Institute Inc., Cary, NC). The replicated cage was considered an experimental unit for all data analysis. The outliers were checked using the UNIVARIATE procedure of SAS. Duncan's multiple range test was conducted to distinguish significant differences among dietary treatments. Significance level for the statistical test was set at $P < 0.05$.

RESULTS AND DISCUSSION

Growth Performance and Breast Meat Quality

The data for growth performance of broiler chickens were presented in Table 5. The PC treatment had a greater ($P < 0.05$) BW and FI than NC treatment in all periods (i.e., 8−21, 22−34, and 8−34 d of age). The Trp biomass treatment had no differences in BW and FI as compared to PC treatment, but had a greater ($P < 0.05$) BW and FI than NC treatment. No differences in FCR and mortality were found among treatments in all periods. Likewise, there were no differences in all meat quality measurements among treatments (Table 6).

Accurate information for essential AA requirements and the extent of AA utilization in diets is mandatory for feed formulation in poultry (Rosa et al., 2001). The Trp is an essential AA for poultry as observed in other animals (Rosa et al., 2001) because of its role in protein synthesis and other pivotal processes in the body (Fouad et al., 2021). Therefore, in the current experiment, birds fed Trp-deficient diets containing 40% less amounts of digestible Trp (i.e., NC treatment) had decreased growth performance compared with those fed the diets containing adequate amounts of Trp from the supply of crystalline Trp or Trp biomass. A similar growth depression of broiler chickens has been reported in the previous studies

Table 5. Effect of dietary Trp biomass on growth performance of broiler chickens from 8 to 34 d of age[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
8−21 d					
BW (g)	792[a]	727[b]	779[a]	11.8	<0.05
BWG (g)	586[a]	521[b]	573[a]	11.8	<0.05
FI (g)	950[a]	866[b]	928[a]	14.4	<0.05
FCR (g/g)	1.62	1.66	1.62	0.020	0.28
Mortality (%)	2.1	1.0	0.0	0.99	0.35
22−34 d					
BW (g)	1,552[a]	1,426[b]	1,520[a]	24.0	<0.05
BWG (g)	760	699	741	19.4	0.10
FI (g)	1,620[a]	1,497[b]	1,573[a]	25.1	<0.05
FCR (g/g)	2.13	2.15	2.13	0.035	0.95
Mortality (%)	3.1	5.2	1.0	1.66	0.23
8−34 d					
BW (g)	1,552[a]	1,426[b]	1,520[a]	24.0	<0.05
BWG (g)	1,346[a]	1,220[b]	1,315[a]	24.0	<0.05
FI (g)	2,560[a]	2,356[b]	2,502[a]	34.2	<0.05
FCR (g/g)	1.90	1.93	1.90	0.019	0.47
Mortality (%)	5.2	6.3	1.0	1.61	0.07

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: BWG, BW gain; FI, feed intake; FCR, feed conversion ratio.

(Carew et al., 1983; Hsia et al., 2005). Interestingly, birds fed Trp-deficient diets had less FI than birds fed diets containing the recommended concentrations of digestible Trp. It is generally appreciated that feeding diets deficient in any AA increases FI of birds in order to fulfill the requirement of the deficient AA (Carew et al., 2003). Therefore, decreased FI by feeding Trp-deficient diets in this experiment was unexpected. The possible reason may be decreased production of serotonin because Trp is a precursor molecule of serotonin that is reported to play a role in stimulating FI of animals (Rosebrough, 1996; Corzo et al., 2005).

Table 6. Effect of dietary Trp biomass on breast meat quality of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
Yield (%)	13.65	13.49	14.39	0.495	0.40
pH (1 h)	6.61	6.63	6.63	0.033	0.81
pH (24 h)	6.32	6.29	6.33	0.041	0.83
WHC (%)	67.57	66.23	70.69	2.223	0.36
TBARS	0.32	0.34	0.33	0.009	0.41
Meat color					
L*	46.58	46.31	46.12	0.543	0.84
a*	6.00	6.07	5.44	0.440	0.55
b*	20.32	23.19	20.58	1.180	0.19

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: Yield, relative breast weight; WHC, water holding capacity; TBARS, thiobarbituric acid reactive substances (malondialdehyde equivalents per g of meat sample); L*, lightness; a*, redness; b*, yellowness.

In the current experiment, dietary Trp biomass was evaluated as an alternative Trp source to conventional crystalline Trp in broiler diets based on various measurements related to possible physiological functions of Trp. Broiler chickens fed diets containing Trp biomass had similar growth performance to those fed diets supplemented with crystalline Trp when digestible Trp concentrations were equalized between 2 diets. Similar results have also been reported for other AA biomass. For example, the supplementation of Thr biomass in broiler diets (0.117% and 0.113% in grower and finisher phases, respectively) showed no differences as compared to feeding diets supplemented with

Table 7. Effect of dietary Trp biomass on stress indicators of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
H:L ratio	0.31[c]	0.51[a]	0.41[b]	0.015	<0.05
Feather CORT (pg/mg)	2.0	1.7	2.2	0.52	0.78

[a-c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: H:L ratio, heterophil to lymphocyte ratio; Feather CORT, feather corticosterone concentrations.

crystalline Thr (Wensley et al., 2020). Likewise, Jespersen et al. (2021) reported that feeding diets containing 0.30% Lys biomass to broiler chickens showed a similar performance to feeding diets fortified with crystalline Lys. Therefore, the current results clearly indicate that Trp biomass used in this experiment can be used as a potential alternative to conventional crystalline Trp in broiler diets.

The AA in the poultry diet are essential to synthesize muscle, and therefore, different sources and amounts of dietary AA may influence meat quality of broiler chickens (Javed, 2020). In the current study, however, breast meat quality including breast meat yield as the percentage of BW, pH, WHC, meat color, and TBARS value was not affected by the sources and concentrations of Trp in diets. The previous studies also reported that different concentrations of Trp in diets had little influence on breast meat quality in broiler chickens (Markus et al., 2000; Kim et al., 2013; Bello et al., 2018). Therefore, it appears that the concentrations of dietary Trp used in this experiment are unlikely to influence breast meat quality of broiler chickens.

Stress Biomarkers and Immune Responses

The PC treatment had less ($P < 0.05$) blood H:L ratio than NC and Trp biomass treatments (Table 7). The Trp biomass treatment had less ($P < 0.05$) blood H:L ratio than NC treatment. However, feather CORT concentrations were not affected by dietary treatments. The values for CBH test did not differ at all measured times among treatments (Table 8).

Dietary Trp has been considered a functional AA to regulate aggressive behavior and stress responses because Trp is the precursor of serotonin and melatonin (Markus et al., 2000; Kim et al., 2013; Bello et al., 2018). In the current experiment, 2 stress biomarkers including blood H:L ratio and feather CORT concentrations were used to assess the effects of different sources and amounts of Trp in broiler diets. Feeding Trp-deficient diets to broiler chickens increased blood H:L ratio compared with feeding diets containing sufficient amounts of Trp from crystalline Trp or Trp biomass, which may indicate that dietary Trp deficiency increases stress responses of broiler chickens. Similar results were observed by Moneva et al. (2008) who reported that additional supplementation of dietary Trp decreased blood H:L ratio in chickens exposed to a nutritional stress. However, despite identical concentrations of digestible Trp between 2 treatment diets, feeding diets supplemented with Trp from Trp biomass

Table 8. Effect of dietary Trp biomass on cutaneous basophil hypersensitivity (CBH) responses of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
0 h (mm)	0.00	0.00	0.00		
6 h (mm)	0.84	0.80	0.61	0.140	0.48
12 h (mm)	0.41	0.52	0.45	0.152	0.87
24 h (mm)	0.26	0.23	0.24	0.063	0.94

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]CBH response is the cutaneous basophil hypersensitivity response, which measured as toe web skin swelling before and after injection with phytohemagglutinin-P.

Table 9. Effect of dietary Trp biomass on jejunal morphology of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
VH (μm)	1,626	1,527	1,618	39.2	0.16
VW (μm)	231	225	218	9.7	0.64
CD (μm)	174[b]	200[a]	177[b]	4.8	<0.05
VH:CD ratio	9.95[a]	8.05[b]	9.76[a]	0.274	<0.05

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: CD, crypt depth; VH, villus height; VW, villus width; VH:CD ratio, villus height to crypt depth ratio.

resulted in a greater blood H:L ratio than feeding diets supplemented with Trp from crystalline Trp. The reason is unclear. However, feather CORT concentrations, which is another potential stress biomarker to a long-term stress in poultry (Lee et al., 2022), were not influenced by different sources and amounts of Trp. A possible reason for this observation may be that nutritional deficiency as a stressor may have limitations to affect feather CORT concentrations. The relatively short period of feeding different sources and amounts of Trp to broiler chickens may be another reason for little effects on feather CORT concentrations.

The CBH test is widely used to measure cell-mediated immune responses in poultry (Allahdo et al., 2018). The CBH test via subcutaneous injection of *Phaseolus vulgaris* lectin P (**PHA-P**) measures thymus-dependent responses that occur through T lymphocytes (Allahdo et al., 2018), and therefore, CBH test determines T-cell activity in the cellular immunity (Allahdo et al., 2018). In the present study, however, the values for CBH test were not affected by different sources and amounts of dietary Trp. Similarly, no significant effects of dietary Trp concentrations on immune responses were observed in the previous study (Sharideh and Zaghari, 2021).

Jejunal Morphology, Antioxidant Status, and Gene Expression

The PC and Trp biomass treatments had less ($P < 0.05$) CD than NC treatment (Table 9). Similarly, PC and Trp biomass treatments had greater ($P < 0.05$) VH:CD ratio than NC treatment. However, Trp biomass treatment had no differences in CD and VH:CD as compared to PC treatment. No differences were observed in VH and VW among treatments.

Antioxidant status such as TAC, MDA, and ROS in the jejunal mucosa was not affected by dietary treatments (Table 10). For the tight junction-related genes in the jejunal mucosa, the expression level of the OCLN for PC treatment had greater ($P < 0.05$) than for NC and Trp biomass treatments (Table 11). However, expression levels of other tight junction-related genes (CLDN-1, ZO-1, and JAM-2) were not influenced by dietary treatments. Likewise,

Table 10. Effect of dietary Trp biomass on antioxidant status in the jejunal mucosa of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
TAC (μmol/mg protein)	831	817	818	34.5	0.95
MDA (μmol/mg protein)	0.76	0.88	1.26	0.203	0.22
ROS (mM)	95.47	96.04	94.13	3.911	0.94

[1]Data are least squares means of 8 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: MDA, malondialdehyde; ROS, reactive oxygen species; TAC, total antioxidant capacity.

Table 11. Effect of dietary Trp biomass on the relative expression of tight junction-related genes in the jejunal mucosa of broiler chickens[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
OCLN	0.50[a]	0.18[b]	0.17[b]	0.063	<0.05
CLDN-1	1.99	1.01	2.01	0.819	0.66
ZO-1	1.01	0.78	1.29	0.310	0.54
JAM-2	0.91	0.64	0.93	0.211	0.56

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 8 observations per treatment. Data represent the relative values of gene expression to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: CLDN-1 claudin-1; JAM-2, junctional adhesion molecule B; OCLN, occludin; ZO-1, zonula occludens-1.

dietary treatment did not affect the expression levels of inflammatory cytokine genes (Table 12).

Table 12. Effect of dietary Trp biomass on the relative expression of inflammatory cytokine genes in the jejunal mucosa of broiler chicken[1].

	Dietary treatment[2]				
Items	PC	NC	Trp biomass	SEM	*P*-value
Pro-inflammatory cytokine[3]					
IFN-γ	1.18	0.45	1.35	0.607	0.57
IL-1β	0.71	0.83	1.02	0.225	0.64
IL-6	1.19	0.78	0.62	0.339	0.52
Anti-inflammatory cytokine[4]					
IL-4	0.30	0.33	0.36	0.097	0.91
IL-10	1.01	2.14	1.37	0.803	0.63
TGF-β_4	1.22	0.78	0.80	0.327	0.60

[1]Data are least squares means of 8 observations per treatment. Data represent the relative values of gene expression to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]IFN-γ, interferon gamma; IL-1β, interleukin 1 beta; IL-6, interleukin 6.

[4]IL-4, interleukin 4; IL-10, interleukin 10; TGF-β_4, transforming growth factor beta-4.

Intestinal VH, CD, and VH:CD ratio are important indicators of intestinal health, recovery, and function (Song et al., 2017). The beneficial effects of high amounts of Trp and protein in diets on the small intestinal structure, especially with increasing VH of the jejunum, have been reported (Ale Saheb Fosoul et al., 2016). In the current study, birds fed Trp-deficient diets had increased CD and concomitantly decreased VH:CD ratio than those fed diets fortified with Trp from crystalline Trp or Trp biomass, indicating that Trp deficiency may induce impairments in intestinal structures. Rao et al. (2021) reported that piglets fed diets containing 0.28% and 0.35% Trp had increased VH and VH:CD ratio in the jejunum and ileum than those fed diets containing 0.14% and 0.21% Trp. Rapidly growing broiler chickens require high amounts of protein in the intestine, and approximately 12% of newly synthesized protein are used in the gastrointestinal tract (Choct, 2009). Therefore, it is suggested that Trp deficiency may have a significantly negative effect on the intestinal development of broiler chickens. In the current experiment, the effects of Trp biomass on the jejunal morphology of broiler chickens were similar to those of crystalline Trp. Therefore, it is likely that Trp biomass may be highly efficient to support the intestinal structure and development to an extent similar to crystalline Trp in broiler chickens.

Dietary Trp has been reported to have the potential to reduce oxidative stress and promote antioxidant capacity in poultry (Patil et al., 2013; Liu et al., 2015; Dong and Zou, 2017). Patil et al. (2013) reported that Trp, as the precursor of serotonin and melatonin, can inhibit oxidative damages in broiler chickens by increasing activity of antioxidant enzymes such as catalase and superoxide dismutase. Similarly, the previous studies also reported increased antioxidant status and catalase activity in the serum, liver, and breast meat of white Pekin ducks fed diets containing additional Trp (Liu et al., 2015). Likewise, increasing supplementation of dietary Trp increased serum total superoxide dismutase (**T-SOD**) activity and TAC contents with decreased MDA content in the serum (Dong and Zou, 2017). In the current study, however, antioxidant status including

TAC, MDA, and ROS in the jejunal mucosa was not affected by different sources and amounts of Trp in diets, possibly because our experimental conditions may be insufficient to induce oxidative stress in broiler chickens. However, if broiler chickens are exposed to high oxidative stress, the considerable reduction in oxidative stress may be occurred by dietary supplementation of Trp.

The tight junction as an intercellular junctional complex plays a role in maintaining the integrity of intestinal barrier functions (Ulluwishewa et al., 2011). The tight junction structure consists of transmembrane proteins, such as CLDN, OCLN, JAM, and intracellular plaque proteins, such as ZO-1 (Furuse et al., 1993, 1998; Citi et al., 1988; Martin-Padura et al., 1998). It has been reported that sufficient amounts of dietary Trp are essential to support the tight junction barrier functions in the intestine of animals (Suzuki, 2020). However, there has been a lack of data pertaining to the effect of different sources and amounts of dietary Trp on intestinal tight junction barrier in poultry. Previous experiments reported that increasing concentrations of Trp in diets increased expressions of tight junction-related genes including ZO-1, ZO-3, and CLDN-1 in the jejunum of weanling pigs (Liang et al., 2019). However, the results have been inconsistent. For instance, in another study, additional supplementation of Trp decreased expressions of tight junction-related genes including OCLN and ZO-1 in the jejunum of weanling pigs (Tossou et al., 2016). In the current experiment, however, different sources and amounts of Trp in diets had no significant effects on the expressions of ZO-1, CLDN-1, and JAM-2 genes. However, feeding Trp-deficient diets or diets supplemented with Trp biomass resulted in decreased expression of OCLN compared with feeding diets supplemented with Trp from crystalline Trp. Despite our findings, the clear reason why only one specific gene related to tight junction barrier was decreased by Trp deficiency or Trp biomass remains unknown.

Inflammatory cytokines regulate innate and adaptive immune functions and the balance of pro-inflammatory (i.e., IFN-γ, IL-1β, and IL-6) and anti-inflammatory cytokines (i.e., IL-4, IL-10, and TGF-β_4) is critical for pathological inflammatory status (Sultani et al., 2012; Pitargue et al., 2019). The expression levels of these cytokines may be affected by the sources and concentrations of proteins and AA in animal diets (Bhanja et al., 2014; Tang et al., 2018). Han et al. (2018) reported that any deficiency of essential AA may have a large impact on the splenic and intestinal expression of cytokine genes. Emadi et al. (2011) reported that Trp may show a positive effect against infectious bursal diseases in chickens by reducing IFN-α and IFN-γ levels in the serum. In the current study, however, gene expressions of inflammatory cytokines in the jejunal mucosa were not affected by different sources and amounts of Trp in diets.

Energy and Nitrogen Utilization in Diets

The GE retention did not differ among 3 treatment diets. However, N retention for PC and Trp biomass treatment diets was greater (*P*

Table 13. Effect of dietary Trp biomass on apparent total tract nitrogen (N) utilization and metabolizable energy in broiler diets (Metabolism trial)[1].

	Dietary treatment[2]				
Items[3]	PC	NC	Trp biomass	SEM	*P*-value
GE retention (%)	79.5	79.6	79.5	0.34	0.97
N retention (%)	48.9[a]	46.9[b]	50.0[a]	0.64	<0.05
AME (kcal/kg)	3,164	3,189	3,183	14.1	0.44
AME_n (kcal/kg)	3,026	3,056	3,042	13.2	0.29

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Data are least squares means of 9 observations per treatment.

[2]PC, positive control; NC, negative control; PC and Trp biomass satisfied digestible Trp requirement, but NC contained 40% less amounts of digestible Trp than its requirement.

[3]Abbreviations: AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolizable energy; GE, gross energy; N, nitrogen .

< 0.05) than for NC diets with no difference being observed between PC and Trp biomass treatment diets (Table 13). The observation for less N retention for NC diets than for other 2 diets is likely a consequence of AA imbalance in NC diets with Trp deficiency. Based on GE and N retentions, AME and AME_n values of treatment diets were calculated, but those values did not differ among treatments. These results indicates that inclusion of Trp biomass has no negative effects on energy utilization in broiler diets.

CONCLUSIONS AND APPLICATIONS

1 Dietary Trp biomass shows similar growth performance, meat quality, immune responses, intestinal health, and utilization of energy and nutrient in diets to conventional crystalline Trp.
2 Therefore, Trp biomass can be used as a potential alternative to crystalline Trp in broiler diets.

DISCLOSURES

Authors declare that there are no competing interests.

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