Gut microbiota and transportation stress response affected by tryptophan supplementation in broiler chickens

Alhassan U. Belloa, Zulkifli Idrusab, Goh Yong Menc, Elmutaz Atta Awada and Abdoreza Soleimani Farjama

aInstitute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Selangor, Malaysia; bDepartment of Animal Science, Universiti Putra Malaysia, Selangor, Malaysia; cDepartment of Preclinical Sciences, Universiti Putra Malaysia, Selangor, Malaysia

ABSTRACT

Stimulation of serotonergic activity by tryptophan (TRP) supplementation is known to influence behavioural and physiological processes. One hundred and twenty male broiler chicks were assigned in groups of 5–24 battery cage and fed experimental diets with 0.22, 0.42 and 0.62% of digestible TRP during 21–42 d. On 42 d, birds were challenged with 2 h of transportation stress and samples were collected before or after the transportation. The results revealed that TRP increased feed intake, but had no significant effect on growth performance. Regardless of transportation, heat shock protein 70 (HSP70) and corticosterone (CORT) decreased and serotonin (5-HT) elevated by increasing TRP level in diet. Breast muscle pH and colour were not affected by elevation in dietary TRP, but drip loss decreased and shear force increased. Quantification of gut microflora showed that supplementation of TRP increased Enterococci, and Bifidobacteria populations, while E. coli, Clostridia, Campylobacter and Enterobacteria populations decreased. The effect of diet on Lactobacilli population was not significant. In conclusion, feeding broilers with higher levels of TRP improves their welfare condition both before and after transportation stress, as measured by lower serum CORT and HSP70 and higher 5-HT. Increasing dietary TRP level may shift the balance of pathogenic/non-pathogenic bacteria in gut to a favourable state.

ARTICLE HISTORY

Received 14 April 2017
Revised 5 June 2017
Accepted 7 June 2017

KEYWORDS
Tryptophan; meat quality; corticosterone; microflora; transportation

Introduction

Finishing broiler chickens are commonly subjected to a range of stressors including catching, handling, crating and transportation. These changes in chicken’s microenvironment activate stress response pathways in a range between mild to severe response depending on the adversity of the condition. Physiologically, these changes may lead to depletion of muscle glycogen reserves and therefore affecting the rate and extent of pH decrease, which could affect meat quality (Debut et al. 2003). On the other hand, stressful experience may disturb the balance of intestinal microbiota and lead to excessive growth of pathogens while decreasing the proportion of beneficial bacteria such as Lactobacilli and Bifidobacteria (Meimandipour et al. 2010; Zhang et al. 2016). Proliferation of Bifidobacteria and Lactobacilli in the gut can increase non-anxious behaviour in mouse through vagus nerve, maybe through bacteria-derived neuroactive elements (Saulnier et al. 2013). Moreover, pathogenic microbiota colonisation poses major risk to poultry health and may negatively affect food safety. A recent survey conducted by the Food Standards Agency (FSA) reported Campylobacter contamination in 70% of fresh, shop-bought chickens in the UK (FSA 2015). Campylobacter, Salmonella and E. coli are among the most prevalent foodborne microbial hazards in raw poultry meat. The most effective and harmless strategy to control these pathogens is competitive exclusion (Schneitz 2005). Members of Lactobacilli and Bifidobacteria genus inhibit growth and development of several pathogenic bacteria by producing lactic and acetic acids as their major metabolic end products and less amounts of succinic, formic acids and ethanol (Alakomi et al. 2000).

Pre- or post-transport nutrient supplementation has been largely used to improve the welfare status of finishing animals going through catching, handling, crating and transportation stress (Zulkifli et al. 2000; Bejai et al. 2014). Working with pigs showed that 5 g/kg supplementation of tryptophan (TRP) reduced...
stressed attributed to transportation (Adeola and Ball 1992). Supplementation of TRP induces serotonergic activity by elevation in serotonin (5-HT) synthesis and thus may affect feed intake (FI), stress response, sleeping patterns and aggressiveness (Lacy et al. 1986; Shea et al. 1990; van Hierden et al. 2004). Low levels of TRP in diet resulted in aggressive pecking, flapping and body curling in chickens (Shea et al. 1990). Moreover, during TRP metabolism, several endogenous antioxidants may produce such as melatonin, 5-hydroxytryptophan and 3-hydroxykynurenine (Christen et al. 1990; Huether et al. 1992). Provision of TRP therefore increases antioxidative activity in body (Liu et al. 2015), controlling formation of reactive oxygen species (ROS). High levels of ROS are known to damage cell membrane integrity and membrane-bound receptors and enzymes. In the gut, TRP may also be directly degraded by microbiota to indole and its various derivates. Indole-producing bacteria are reported to inhibit growth and survival of non-indole-producing bacteria (Smith and Macfarlane 1997). Thus, we hypothesise that provision of dietary TRP may reduce the adverse effects of transportation stress and bacterial load of the major foodborne pathogens and therefore improve poultry welfare and meat quality.

Materials and methods

Animal ethics

This study was conducted in accordance with the Universiti Putra Malaysia Research Policy on Animal Ethics and Welfare.

Birds and housing

A total of 120 day-old male broiler chicks (Cobb 500) were purchased from a local hatchery. On arrival, all the chicks were weighed, neck tagged (PN # 7-60-9024-01, Ketchum Manufacturing Inc., Brockville, Ontario, Canada) and randomly assigned in groups of 5–24 battery cage (height × width × length, 45 × 60 × 120 cm) with wire floor (720 cm²/per bird). The cages were located in a conventional open-sided house and ad libitum feed and water, and continuous light were provided.

Dietary treatments

Birds were provided starter diet (3030 kcal of ME/kg; 22% CP) from 1 to 20 d and finisher diet (3180 kcal of ME/kg; 19% CP) from 21 to 42 d. The finisher diet was prepared as three isonitrogenous and isocaloric experimental diets containing varying levels of digestible TRP (0.22, 0.42 and 0.62%; Table 1). Each experimental diet was allocated to eight random cages of birds. The experimental diets were formulated using digestible TRP and its ratio to large neutral amino acids (valine, leucine, isoleucine, tyrosine and phenylalanine). This was to prevent neutralisation effects of TRP by LNAA as they compete for the same transporters in brain (Fernstrom 2013).

Growth performance

Weight gain (WG) and FI were measured for the period of 21–42 d and feed conversion ratio (FCR) was calculated.

Transportation and sampling

On 42 d, at 8:00 blood samples (2 ml) were drawn randomly from wing vein of one bird per cage. The average time between initial capture and end of blood sampling was 1:20 min and should have no influence on corticosterone (CORT) (Romero and Reed 2005). Another two birds per cage were randomly caught and crated (0.80 × 0.60 × 0.31 m) at 10 birds per crate and loaded to an open truck and transported for 2 h between 8:00 and 10:00 a.m. with an average speed of 80 km/h. The journey covered highways, roads with heavy traffic, and traffic lights. At the time of

| Table 1. Composition of experimental diets. |
|---------------------------------------------|
| Ingredients, %                              |
| Corn                                        | 61.10 | 61.47 | 62.05 |
| Soybean meal                                | 30.00 | 29.60 | 29.00 |
| Palm oil                                    | 5.40  | 5.20  | 5.00  |
| Dicalcium phosphate                         | 1.40  | 1.40  | 1.40  |
| Limestone                                   | 1.10  | 1.10  | 1.10  |
| Salt (NaCl)                                 | 0.40  | 0.40  | 0.40  |
| Choline Cl-70%                               | 0.05  | 0.05  | 0.05  |
| Vitamin premixa                             | 0.05  | 0.05  | 0.05  |
| Mineral premixb                             | 0.10  | 0.10  | 0.10  |
| L-lysine                                    | 0.20  | 0.21  | 0.22  |
| α-Methionine                                | 0.16  | 0.16  | 0.16  |
| L-Tryptophan                                | 0.04  | 0.26  | 0.47  |
| Calculated composition                      |       |       |       |
| ME, kcal/kg                                 | 3188  | 3187  | 3189  |
| Crude protein, %                            | 19.04 | 19.08 | 19.04 |
| Digestible lysine, %                        | 0.98  | 0.98  | 0.98  |
| Digestible methionine, %                    | 0.40  | 0.40  | 0.40  |
| Digestible tryptophan, %                    | 0.22  | 0.42  | 0.62  |
| Digestible TRP/LNAA, %                      | 0.050 | 0.100 | 0.150 |
| Calcium, %                                  | 0.82  | 0.82  | 0.82  |
| Nonphytate P, %                             | 0.39  | 0.39  | 0.39  |

aSupplied per kilogram of total diet: vitamin A (retinol), 10,000 U; vitamin D (cholecalciferol), 3000 U; vitamin E (α-tocopherol acetate), 20 U; vitamin K6 (menadione sodium bisulphate), 2.65 mg; vitamin B5 (thiamine mononitrate), 2 mg; vitamin B3 (riboflavin), 8 mg; vitamin B12, 0.025 mg; biotin, 0.2 mg; nicotinic acid, 50 mg; folic acid, 1 mg; γ-pantothenic acid, 20 mg.

bSupplied per kilogram of total diet: Cu (CuSO4), 10 mg; Fe (FeSO4), 60 mg; Zn (ZnO), 60 mg; Mn (MnSO4), 80 mg; Se (NaSeO3), 0.3 mg; I (KI).

cTRP/LNAA: tryptophan to large neutral amino acids ratio.
transportation, the ambient temperature was 28–30 °C. Immediately upon arrival, blood samples were collected from eight birds per crate at random through wing vein. The birds were then slaughtered humanely according to halal method (Farouk et al. 2014) and caecum content samples were collected immediately and stored at –80 °C for bacterial quantification. The right pectoralis major muscle was dissected from the carcase and divided along the long axis into four parts of approximately 30 g each. Each part was labelled, vacuum packaged and stored at 4 °C chiller for determination of pH, drip loss, cooking loss, colour and shear force at 24 h post-mortem. Blood samples were centrifuged at 4000 g for 20 min and serum samples were separated and stored at –20 °C for further analysis of 5-HT, CORT and heat shock protein 70 (HSP70).

**Meat quality**

The breast muscle pH was measured individually at 10 min (pH10 min) and 24 h (pH24 h), post-mortem by the aid of portable digital pH metre (Accumet™ AP115, Fisher Scientific™, Hampton, NH). Each meat sample was tested three times, and the means were used in statistical analysis. For drip loss, approximately 30 g of tissue paper, reweighed and percentage of drip loss was calculated. The meat colour (L*: lightness, a*: redness and b*: yellowness) was determined using an automatic ColorFlex system (Hunt Associates Laboratory, Reston, VA) after 24 h post-mortem. To determine the shear force, approximate 30 g samples were vacuum packaged and subsequently submersed in 80 °C water bath until the core temperature of the muscles reached 78 °C. Samples were removed from the water bath and allowed to cool down to room temperature. The samples were cut into blocks (two blocks per sample) of 10 mm (width), 10 mm (height) and 15 mm (length). The shear force values were determined using a Volodkevitch bite jaw fitted to a texture analyser (TAHD plus®, Stable Micro Systems, Surrey, UK).

**Bacterial quantification**

The population of selected bacteria were determined in caecal samples using quantitative real-time PCR method. DNA was extracted from caecal samples using QiAamp™ Fast DNA Stool Mini Kit (Qiagen Inc., Valencia, CA) in accordance with manufacturer’s protocols. Real-time PCR was carried out using BioRad CFX96 Touch (BioRad, Hercules, CA). Each PCR reaction (25 μl) comprised of 12.5 μl Maxima SYBER Green qPCR Master Mix (Thermofisher Scientific, Waltham, MA), 9.5 μl of nuclease-free H2O, 1 μl of each DNA extract and 1 μl of each primer (for each bacteria). The primer sequences and expected sizes of amplified fragments are shown in Table 2. The reaction conditions for amplification of DNA were 94 °C for 5 min, 40 cycles of 94 °C for 20 s, 60 °C (for *Bifidobacteria*) or 58 °C (for *Lactobacilli*) or 50 °C (for other target bacteria) for 30 s, and 72 °C for 20 s. To confirm the amplification specificity, melting curve analysis was executed after the last cycle of each amplification. Standard curves were constructed using serial dilutions of the PCR products of DNA extracted from each bacteria pure culture. PCR products were purified using the MEGA quick-spin™ kit (iNTRON Biotechnology Inc., Seoul, South Korea) and the DNA purity and concentration in each sample were measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA). The DNA copy numbers of each template per ml of elution buffer were calculated using the formula available online (http://cels.uri.edu/gsc/cndna.html) and then converted to cells/g of digesta.

| Table 2. Primers used in real-time PCR. | PCR product size, bp | Annealing temperature, °C |
|----------------------------------------|----------------------|--------------------------|
| **Enterococci**                         | F-S'-CCCTTATTTGTAATTGCCATCATT-3' | 144                      | 50 |
|                                        | R-S'-ACTGTTGTAATCCACCTGT-3'       |                          |  |
| **Bifidobacteria**                     | F-S'-GGGTGTTGTAATCCACCTGT-3'      | 440                      | 60 |
|                                        | R-S'-TAAGCCCATGGAATTCACCAC-3'      |                          |  |
| **Lactobacilli**                       | F-S'-CATCCAGITGCAAAACCTAAGAG-3'    | 341                      | 58 |
|                                        | R-S'-GATCCGGCTTGCTTGCA-3'         |                          |  |
| **Escherichia coli**                   | F-S'-GTGTGATATCTACCGGCTTCCG-3'     | 82                       | 50 |
|                                        | R-S'-AGAACGCTTTTGTGTAATACAGGA-3'   |                          |  |
| **Clostridia**                         | F-S'-GAATAATGTAATCCAAAACAA-3'      | 196                      | 53 |
|                                        | S-CGAATATCTACCTATCGGATC-3'        |                          |  |
| **Enterobacteria**                     | F-S'-CATGAGCTTTACCAGGAGAAGGC-3'    | 195                      | 50 |
|                                        | R-S'-CTCTACGAGACTCAACGGTTGCA-3'    |                          |  |
| **Campylobacter**                      | F-S'-GACCAAAACCAACCAACGGCAGACCA-3' | 749                      | 55 |
|                                        | R-S'-CTAAGGGGACCAAAAGATGAGG-3'     |                          |  |
**Determination of HSP70**

Concentrations of HSP70 were measured using HSP70 high sensitivity ELISA kit (Catalog # ADI-EKS-715, ENZO Life Sciences Inc., Farmingdale, NY). The protocol of analysis was according to manufacturer’s recommendations. Inter- and intra-assay precisions were <2.63% and <2.19%, respectively. The sensitivity of the assay detection was 0.09 ng/ml (90 pg/ml).

**Determination of 5-HT**

Concentration of 5-HT in the serum was measured using ELISA kit (Catalog # ADI-900-175; ENZO Life Sciences Inc., Farmingdale, NY). The protocol of analysis was according to manufacturer’s recommendations. Inter-assay and intra-assay precisions were <53.9% and <53.8%, respectively. The sensitivity of the assay was determined to be 0.293 ng/ml.

**Determination of CORT**

The CORT was measured by a commercial high sensitivity EIA kit (AC-15F1, IDS, Boldon, UK). The intra- and interassay variabilities were less than 6.7% and 7.8%, respectively; and the detection limit was 27 ng/ml. The protocol of analysis was according to manufacturer’s recommendations.

**Statistical analysis**

The data were analysed as one-way ANOVA using SAS software package (V 9.3, SAS Institute Inc., Cary, NC). Means were separated by Duncan’s multiple range tests. Statistical significance was considered as \( p \leq 0.05 \).

**Results**

The effects of diet on WG, FI and FCR during 21–42 d are presented in Table 3. The results showed that dietary TRP/LNAA had no significant effect on WG and FCR of broiler chickens kept at this period. However, dietary TRP/LNAA significantly increased FI.

| Diet (TRP, %) | WG (g)      | FI (g)      | FCR          |
|--------------|-------------|-------------|--------------|
| 0.22         | 1199 ± 27   | 2073 ± 34   | 1.70 ± 0.05  |
| 0.42         | 1265 ± 36   | 2206 ± 36   | 1.74 ± 0.03  |
| 0.62         | 1274 ± 34   | 2182 ± 51   | 1.72 ± 0.03  |

Probabilities

ANOVA 0.207 0.008 0.750

*Means ± SEM with no common superscripts in a column-subgroup differ significantly (\( p \leq 0.05 \)).

**Discussion**

In the present study, increasing TRP level increased FI when the level was higher than 0.42 and higher. It is not clear how feeding behaviour is regulated by TRP supplementation. Corzo et al. (2005) indicated that the chickens fed low TRP diet displayed unusual behaviour characterised by feed spillage from the feeders and contamination of nearby water containers with the floor litters. It has been reported that intracerebroventricular (ICV) or intraperitoneal injection of 5-HT increases corticotropin-releasing factor (CRF) and therefore induces a potent decrease in FI in chickens (Denbow et al. 1999). This is in contrast with the present observation in TRP supplemented chicks where FI increased along with 5-HT. It is possible that some other pathways of TRP metabolism are involved in regulation of FI. However, the elevation in FI did not result in significant improvement in WG. This may at least partly cause by the effect of TRP on gut motility and peristalsis (Grider and Piland 2007). Digestion and absorption of nutrients are known to be influenced by speed of gut motility and peristalsis (Oosterbosch et al. 1993). It is noteworthy to mention that the observed performance in the current study was lower than the expectations from this strain of broiler chicks. Previous studies also reported such inferior performance in broiler chicks exposed to hot humid tropical condition (Yodseranee and Bunchasak 2012).

Interestingly, the present findings found that drip loss and shear force of the breast muscle significantly affected by dietary TRP. The drip loss decreased and...
Table 4. Influence of diet supplemented with tryptophan (TRP) on meat quality characteristics of broiler chickens subjected to 2 h transportation age 42 d.

| Diet (TRP, %) | pH$_{10 \text{ min}}$ | pH$_{24 \text{ h}}$ | Drip loss, % | Colour | Shear force, N |
|--------------|-----------------|----------------|--------------|--------|---------------|
|              | pH$_{10 \text{ min}}$ | pH$_{24 \text{ h}}$ | Drip loss, % |        |               |
| 0.22         | 6.18 ± 0.05     | 5.85 ± 0.04    | 6.30 ± 0.23$^a$ | 51.06 ± 1.09 | 14.640 ± 0.57 | 84.42 ± 5.84$^c$ |
| 0.42         | 6.11 ± 0.04     | 5.85 ± 0.06    | 4.95 ± 0.29$^b$ | 51.38 ± 0.91 | 13.81 ± 0.51 | 96.41 ± 2.68$^b$ |
| 0.62         | 6.09 ± 0.04     | 5.83 ± 0.02    | 3.41 ± 0.26$^c$ | 53.19 ± 0.74 | 14.18 ± 0.36 | 111.52 ± 3.64$^a$ |
| Probabilities|                 |                |              |        |               |
|              | ANOVA 0.243     |                |              | 0.240  | 0.891         | 0.432            |

L*: lightness; a*: redness; b*: yellowness. $^{a,b,c}$Means ± SEM with no common superscripts in a column-subgroup differ significantly ($p \leq .05$).

Table 5. Mean (±SEM) serum levels of heat shock protein 70 (HSP70), corticosterone (CORT) and serotonin (5-HT) by supplementing diet with tryptophan (TRP) in broiler chickens.

| Diet (TRP, %) | Before transportation | After transportation |        |
|--------------|----------------------|---------------------|--------|
|              | HSP70, ng/ml         | S-HT, ng/ml         | CORT, ng/ml |
| 0.22         | 2.71 ± 0.14$^a$     | 1.05 ± 0.10$^b$    | 0.89 ± 0.05$^b$ |
| 0.42         | 15.52 ± 0.16$^c$    | 32.27 ± 0.37$^b$   | 0.89 ± 0.05$^b$ |
| 0.62         | 2.54 ± 0.19$^a$     | 39.86 ± 1.50$^c$   | 0.54 ± 0.03$^c$ |
|              | 2.54 ± 0.19$^a$     | 39.86 ± 1.50$^c$   | 0.54 ± 0.03$^c$ |
|              | 2.67 ± 0.12$^a$     | 1.91 ± 0.09$^a$    | 0.98 ± 0.07$^a$ |
|              | 16.69 ± 0.29$^a$    | 34.33 ± 0.68$^b$   | 48.75 ± 2.32$^a$ |
|              | 1.59 ± 0.09$^a$     | 0.86 ± 0.01$^a$    | 0.68 ± 0.03$^a$ |

$^{a,b,c}$Means ± SEM with no common superscripts in a column-subgroup differ significantly ($p \leq .05$).

Table 6. Effect of dietary tryptophan (TRP) on caecal microbial populations in 42 d old broiler chickens (cells/g digesta).

| Diet (TRP, %) | Enterococci $\times 10^9$ | Bifidobacteria $\times 10^9$ | Lactobacilli $\times 10^9$ | E. coli $\times 10^9$ | Clostridia $\times 10^9$ | Enterobacteri $\times 10^9$ | Campylobacter $\times 10^9$ | p Value |
|--------------|--------------------------|-----------------------------|--------------------------|------------------------|--------------------------|-------------------------|---------------------------|---------|
| 0.22         | 3.54 ± 0.10$^a$         | 6.73 ± 0.07$^a$             | 5.66 ± 0.10$^a$         | 3.92 ± 0.12$^a$        | 6.71 ± 0.09$^a$         | 3.34 ± 0.07$^a$         | 5.54 ± 0.03$^a$            | <.0001  |
| 0.42         | 4.17 ± 0.14$^a$         | 6.89 ± 0.04$^a$             | 5.71 ± 0.12$^a$         | 3.29 ± 0.11$^a$        | 6.37 ± 0.09$^a$         | 2.42 ± 0.07$^a$         | 4.72 ± 0.03$^a$            | <.0001  |
| 0.62         | 4.52 ± 0.23$^a$         | 7.59 ± 0.11$^a$             | 6.03 ± 0.14$^a$         | 2.04 ± 0.10$^a$        | 6.11 ± 0.07$^a$         | 1.58 ± 0.04$^a$         | 3.85 ± 0.04$^a$            | <.0001  |

$^{a,b,c}$Means ± SEM with no common superscripts in a row differ significantly ($p \leq .05$).

shear force increased in dose dependent manner by TRP supplementation. Previous study by Liu et al. (2015) indicated the same response in duck supplemented with dietary TRP of 0.18–1.08%. However, Wang et al. (2014) failed to observe such effects by TRP supplementation. The discrepancies may be explained by low level of TRP supplementation in study by Wang et al. (2014). The mechanism behind the changes in drip loss and shear force might come from the effects of TRP on antioxidative activity. It is well documented that TRP and its metabolites (e.g. 5-hydroxytryptophan, 3-hydroxykynurenine, melatonin) have antioxidative activity (Christen et al. 1990; Huether et al. 1992). Evidently, in study by Liu et al. (2015), increasing dietary TRP from 0.18 to 1.08% enhanced antioxidative activities (total antioxidant capacity, glutathione peroxidase and catalase) in serum. As a consequence, a smaller extracellular space will be created between adjacent fibres due to fibre shrinkage (Bowker and Zhuang 2015). In other words, myofibrillar structural integrity will be maintained with less denaturation of sarcoplasmic proteins and thus stronger water holding capacity and denser meat structure. It seems that dietary TRP plays a role in decreasing the incidence of PSE (pale, soft, exudative) meat in stressed birds particularly those under transportation stress.

As expected in current experiment, serum 5-HT increased proportional to TRP level. Many studies have shown that increasing 5-HT synthesis is beneficial in alleviating adverse effects of stress animals (Koopmans et al. 2006; Wang et al. 2014; Liu et al. 2015). Moreover, the current result showed that serum HSP70 and CORT decreased in a dose-dependent manner to TRP level. HSP 70 has widely used as indicator of various stress types in chicken (Soleimani et al. 2012a, 2012b). Heat shock proteins protect organisms from the toxic effects of stressful environment by playing role in assembly and disassembly of protein complexes, folding and unfolding of proteins and refolding of damaged proteins. This is the first report of such phenomenon. The exact mechanism behind reduction of HSP70 by elevation of TRP is not clear, however, it may probably be explained by antioxidative activity following TRP metabolism. Melatonin, 5-hydroxytryptophan and 3-hydroxykynurenine are among the main metabolites of TRP with antioxidative properties (Christen et al. 1990; Huether et al. 1992). Herichova et al. (1998) reported that oral administration of TRP increased melatonin synthesis in chicken. Liu et al. (2015) showed that increasing dietary TRP resulted in enhancement of total antioxidant capacity, glutathione peroxidase, and catalase activity in serum. On the other hand, one of the main inducing factor of HSP70 expression is formation of ROS. Low levels of intracellular ROS play a major role in redox signalling, but in high amounts, ROS cause lipid peroxidation,
compromise the cell membrane integrity and deactivate the membrane-bound receptors and enzymes. It is therefore speculated that elevation of dietary TRP resulted in higher antioxidative activity and thus reducing ROS and in turn HSP70. Regarding the possible mechanism behind the reduction of CORT by dietary TRP level, existence of functional relationship between serotonergic system and hypothalamic-pituitary-adrenal (HPA) axis can be mentioned. Study by Chen and Miller (2012) reported that suprachiasmatic nucleus (SCN) receive a dense 5-HT fibre innervation arising from midbrain, and 5-HT acts as an inhibitory neurotransmitter that inhibits CORT secretion by modulating the adrenal sensitivity to adrenocorticotropic hormone (ACTH) via the SCN-sympathetic nervous system-adrenal (SCN-SNS-adrenal) pathway. This may cause adrenal hypotrophy (Tanke et al. 2008) and feedback inhibition of CORT secretion. However, studies by Mench (1991) and van Hierden et al. (2004) indicated a higher CORT level in Trp supplemented birds. The reason for such discrepancy is not clear, but it may be attributed to very high level of Trp in these studies (1.585 and 2%, respectively). Long-term usage of high levels of Trp or 5-HT showed to have toxic effects in body (Zhang et al. 2009).

The most interesting part of the current results is that dietary TRP level has prebiotic-like role in chicken. Quantification of selected species of bacteria belonging to pathogenic or non-pathogenic group showed that non-pathogenic bacteria (Bifidobacteria and Enterococci) greatly increased along with decrease in pathogenic ones (E. coli, Clostridia, Enterobacteria and Campylobacter). This is a strong prebiotic-like effect as such reported with usage of known prebiotics (Guo et al. 2004; Abudabos et al. 2015). To the best of our knowledge, this is the first study reporting such effect for dietary TRP. The exact reason behind the observation is not clear and further multidisciplinary studies needed to shed light on the issue. However, some probable causes can be speculated. The gut bacteria can directly utilise TRP and produce indole (Lee and Lee 2010; Kim and Park 2015). The formation of indole is catalysed by the enzyme tryptophanase, which is inducible by dietary TRP presence (Smith and Macfarlane 1997). Many bacteria may use the indolic compounds to thrive over other bacteria in multispecies communities. Indole producing bacteria reported to inhibit the growth and survival of none-indole-producing bacteria such as Enterobacteria, especially within the genera Salmonella and Shigella (Smith and Macfarlane 1997). Nowak and Libudzisz (2006) also reported the same growth inhibitory effects for indolyl acetic acid added to Lactobacilli culture. The results of this study may explain the lack of significant effect of diet on Lactobacilli in the current study. It is noteworthy to mention that, reducing pathogenic bacteria such as E. coli, Clostridia, Enterobacteria and Campylobacter not only improve chicken’s health and well-being, it may also benefit safety of food industry, as these bacteria are among the major causes of food-borne diseases.

In conclusion, increasing dietary TRP level to 0.42% or higher may improve FI and meat quality in broiler chickens and shift the balance of pathogenic/non-pathogenic bacteria to a favourable state. Chickens fed with 0.42% or higher levels of dietary TRP have better welfare status before or after transportation stress, as measured by lower serum CORT and HSP70 and higher 5-HT. In a cautionary note, it should be mentioned that the broiler chickens, in this study, were raised in a cage system with wire floor and the effectiveness of dietary TRP level should be tested in other rearing systems as well.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This research was funded by the Universiti Putra Malaysia (Grant Putra Scheme, GP-IPS 9429000), and Ministry of Higher Education, Malaysia (Long Term Research Grant Scheme, LRGS 2/2012).

References
Abudabos AM, Al-Batshan HA, Mursheed MA. 2015. Effects of prebiotics and probiotics on the performance and bacterial colonization of broiler chickens. S Afr J Anim Sci. 45:419–428.
Adeola O, Ball RO. 1992. Hypothalamic neurotransmitter concentrations and meat quality in stressed pigs offered excess dietary tryptophan and tyrosine. J Anim Sci. 70:1888–1894.
Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. 2000. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol. 66:2001–2005.
Bejaei M, Bennett DC, Schaefer AL, Cheng KM. 2014. Effects of pre-transport nutrient supplementation and transport duration on the post-transport blood biochemistry, body weight and welfare of ostriches. Anim Welfare. 23:209–217.
Bowker B, Zhuang H. 2015. Relationship between water-holding capacity and protein denaturation in broiler breast meat. Poult Sci. 94:1657–1664.
Chen GL, Miller GM. 2012. Advances in tryptophan hydroxylase-2 gene expression regulation: new insights into serotonin–stress interaction and clinical implications. Am J Med Genet B: Neuropsych Genet. 159:152–171.
Christen S, Peterhans E, Stocker R. 1990. Antioxidant activities of some tryptophan metabolites: possible implication
for inflammatory diseases. Proc Natl Acad Sci USA. 87:2506–2510.

Corzo A, Kidd MT, Thaxton JP, Kerr BJ. 2005. Dietary tryptophan effects on growth and stress responses of male broiler chicks. Br Poult Sci. 46:478–484.

Debut M, Berri C, Baeza E, Sellier N, Arnould C, Guemene D, Jehl N, Boutten B, Jego Y, Beaumont C, et al. 2003. Variation of chicken technological meat quality in relation to genotype and pre-slaughter stress conditions. Poult Sci. 82:1829–1838.

Denbow DM, Snapir N, Furuse M. 1999. Inhibition of food intake by CRF in chickens. Physiol Behav. 66:645–649.

Farouk MM, Al-Mazeedi HM, Sabow AB, Bekhit AED, Adeyemi KD, Sazili AQ, Ghani A. 2014. Halal and kosher slaughter methods and meat quality: a review. Meat Sci. 98:505–519.

Fernstrom JD. 2013. Large neutral amino acids: dietary effects on brain neurochemistry and function. Amino Acids. 45:419–430.

FSA. 2015. [accessed 2017 April 13]. https://www.food.gov.uk/sites/default/files/campylobacter-retail-survey-final-report.pdf.

Grider JR, Piland BE. 2007. The peristaltic reflex induced by short-chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF. Am J Physiol Gastrointestinal Liver Physiol. 292:G429–G437.

Guo FC, Williams BA, Kwakkel RP, Li HS, Li XP, Luo JY, Li WK, Verstegen MWA. 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult Sci. 83:175–182.

Herichova I, Zeman M, Veselovsk J. 1998. Effect of tryptophan administration on melatonin concentrations in the pineal gland, plasma and gastrointestinal tract of chickens. Acta Vet (Brno). 67:89–95.

Huether G, Poeggeler B, Reimer A, George A. 1992. Effect of tryptophan administration on circulating melatonin levels in chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. Life Sci. 51:945–953.

Kim J, Park W. 2015. Indole: a signalling molecule or a mere metabolic product by which alters bacterial physiology at a high concentration? J Microbiol. 53:421–428.

Koopmans SJ, Guzik AC, van der Meul en J, Dekker R, Kogut J, Kerr BJ, Southern LL. 2006. Effects of supplemental l-tryptophan on serotonin, cortisol, intestinal integrity, and behavior in weanling piglets. J Anim Sci. 84:963–971.

Lacy MP, Van Krey HP, Skews PA, Denbow DM. 1986. Tryptophan’s influence on feeding and body temperature in the fowl. Poult Sci. 65:1193–1196.

Lee JH, Lee J. 2010. Indole as an intercellular signal in microbial communities. FEMS Microbiol Rev. 34:426–444.

Liu Y, Yuan JM, Zhang LS, Zhang YR, Cai SM, Yu JH, Xia ZF. 2015. Effects of dietary tryptophan supplementation on growth performance, antioxidative activity, and meat quality of ducks under high stocking density. Poult Sci. 94:1894–1901.

Meimandipour A, Soleimani AF, Hair-Bejo M, Shuhaimi M, Azhar K, Nategli L, Rasti B, Yazid AM. 2010. Efficacy of lactobacilli to normalize production of corticosterone induced by unpleasant handling of broilers. S Afr J Anim Sci. 40:327–333.

Mench JA. 1991. Research note: feed restriction in broiler breeders causes a persistent elevation in corticosterone secretion that is modulated by dietary tryptophan. Poult Sci. 70:2547–2550.

Nowak A, Libudzisz Z. 2006. Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. Anaerobe. 12:80–84.

Oosterbosch L, von der Ohe M, Valdivovina MA, Kost LJ, Phillips SF, Camilleri M. 1993. Effects of serotonin on rat ileocolonic transit and fluid transfer in vivo: possible mechanisms of action. Gut. 34:794–798.

Romero LM, Reed JM. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? Comp Biochem Physiol A Mol Integr Physiol. 140:73–79.

Saulnier DM, Ringel Y, Heyman MB, Foster JA, Bercik P, Shulman RJ, Versalovic J, Verdu EF, Dinan TG, Hecht G, et al. 2013. The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. Gut Microb. 4:17–27.

Schneitz C. 2005. Competitive exclusion in poultry—30 years of research. Food Contr. 16:657–667.

Shea MM, Mench JA, Thomas OP. 1990. The effect of dietary tryptophan on aggressive behavior in developing and mature broiler breeder males. Poult Sci. 69:1664–1669.

Smith EA, Macfarlane GT. 1997. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. Microb Ecol. 33:180–188.

Soleimani AF, Zulkifli I, Hair-Bejo M, Omar AR, Raha AR. 2012b. The role of heat shock protein 70 in resistance to Salmonella enteritidis in broiler chickens subjected to neonatal feed restriction and thermal stress. Poult Sci. 91:340–345.

Soleimani AF, Zulkifli I, Omar AR, Raha AR. 2012a. The relationship between adrenocortical function and hsp70 expression in socially isolated Japanese quail. Comp Biochem Physiol Biochem Mol Biol. 161:140–144.

Tanke MAC, Alsera S, Doornbos B, van der Most PJ, Goeman K, Postema F, Korf J. 2008. Low tryptophan diet increases stress-sensitivity, but does not affect habituation in rats. Neurochem Int. 52:272–281.

van Hideren YM, Koohass J, Korte SM. 2004. Chronic increase of dietary l-tryptophan decreases gentle feather pecking behaviour. Appl Anim Behav Sci. 89:71–84.

Wang B, Min Z, Yuan J, Zhang B, Guo Y. 2014. Effects of dietary l-tryptophan and stocking density on the performance, meat quality, and metabolic status of broilers. J Anim Sci Biotechnol. 5:44.

Yodseraneet R, Bunchasak C. 2012. Effects of dietary methionine source on productive performance, blood chemical, and hematological profiles in broiler chickens under tropical conditions. Trop Anim Health Prod. 44:1957–1963.

Zhang G, Krishnamoorth S, Ma Z, Yukovich NP, Huang X, Tao R. 2009. Assessment of 5-hydroxytryptamine efflux in rat brain during a mild, moderate and severe serotonin-toxicity syndrome. Eur J Pharmacol. 615:66–75.

Zhang P, Yan T, Wang X, Kuang S, Xiao Y, Lu W, Bi D. 2016. Probiotic mixture ameliorates heat stress of laying hens by enhancing intestinal barrier function and improving gut microbiota. Ital J Anim Sci. 16:292–300.