Effects of *Trichoderma* fermented wheat bran on growth performance, intestinal morphology and histological findings in broiler chickens

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**ABSTRACT**

This experiment investigated the effects of wheat bran fermented with *Trichoderma pseudokonigii* (FWB) on growth performance, intestinal morphology and the incidence of non-specific pathological lesions in broilers. In vitro experimental results showed that cellulase and xylanase activity of fermented wheat bran reached its peak at day 4 and solid-state fermentation (SSF) enhanced the reducing sugar content. In addition, the possibility of *Trichoderma* adhering to the broiler’s crop epitheliums was also discovered. A total of 180 day-old Ross 308 male broilers were randomly distributed into one of the three dietary groups until 35 d of age: basal diet (control), 10% of basal diet replaced with wheat bran (10% WB) or 10% of diet replaced with fermented wheat bran (10% FWB). Results showed that 10% FWB group had lower feed consumption than the others, but improved feed conversion ratio (FCR) for starter phase (1 to 21 d) when compared to the control group. Furthermore, 10% FWB group had significantly increased villus height and villus height/crypt depth ratio in the ileum compared to the control group. *Coliform* bacteria count in the ileum was lower in the 10% WB group than the control, however, there were no differences between the 10% WB and 10% FWB treatment groups in the *C. perfringens* count. There were no morphological changes or incidences of non-specific pathological lesions in the 10% FWB group. These results suggested that replacing 10% of a basal diet with fermented wheat bran could not only improve growth performance but also provide optimal intestinal morphology in broilers.

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**Introduction**

In recent years, due to the energy crisis, grain crops have been used for biomass energy, resulting in increased prices for the main raw materials in animal feed. The solution to the feed shortage could be the use of alternative crops or agricultural by-products (Oguri et al. 2013). Compared to the major grain crops, agricultural by-products are inexpensive, but largely useless since their abundant fibre content limits their use as feed for monogastric animals.

Wheat bran (WB) is the main by-product of wheat flour processing. Souci and Kirchhoff (2008) reported that WB contains a high amount of total dietary fibre (451 g/kg) and other relevant compounds, including protein (160 g/kg), fat (47 g/kg), carbohydrates (177 g/kg) and minerals (61.5 g/kg). However, it is well known that the increased fibre content (likely non-starch polysaccharides, or NSP) negatively correlates with animal feed intake as well as digestibility, ultimately affecting growth and production performance. Therefore, the use of wheat bran has been limited in animal feed (Slominski et al. 2004; Nortey et al. 2007).

In recent years, however, scientists have begun to use fungal inoculum for the fermentation of agricultural by-products in order to raise their nutritional value (Sharma & Arora 2011). *Trichoderma* species are known for producing cell wall-degrading enzymes, which can be used for both industrial productions and as biocontrol agents to inhibit the activity of soil borne pathogens (Harman et al. 2004). *Trichoderma* species also possesses high lignocellulosic-degrading capabilities and may further degrade the lignocellulosic biomass for use in animal feed. Hatta et al. (2014) showed that broilers supplemented with 15% *Trichoderma viride*-fermented copra meal had the same growth rate as the control group. Similarly, Odeniyi et al. (2012) indicated that dietary supplementation with *Trichoderma virens*-fermented palm-fruit husk had no significant effect on body weight or egg weight in...
layers. Aderolu et al. (2007) pointed out that rice husks fermented with *Trichoderma viride* demonstrated both improved energy content and greater degradation of lignocellulosic content compared to non-fermented materials. Omwango et al. (2013) reported that fermented pineapple waste with *Trichoderma viride* increased both the crude protein and ash content, while simultaneously reduced the crude fibre content. Rostika and Safitri (2012) examined the use of corn cobs fermented by *Trichoderma viride, Trichoderma reesei, Aspergillus oryzae* and *Rhizopus oligosporus* as material feed for java barb to further increase the growth rate of the fish. Mohamed and Abou-Zeina (2008) also reported that supplementation with *Trichoderma viride*-fermented sugar beet pulp in a concentrated feed mixture for goat kids increased the nutritive value of the rations. Furthermore, it had positive effects on performance and metabolic hormones without any adverse effects on serum biochemistry values. However, few studies have reported on the addition of *Trichoderma* fermented wheat bran to the broiler diet. Therefore, this study was conducted to assess its effects on the growth performance, intestinal microbiota and intestinal morphology and histology of broiler chickens.

**Materials and methods**

**Microorganisms, substrate procurement and inoculums**

*Trichoderma pseudokoningii* was used for fermentation; it was procured from the Department of Biotechnology, National Formosa University, Yunlin, Taiwan. The organisms were cultivated on Potato-Dextrose Agar (PDA) at 25 °C for 1 week. Some broth was added to each disc before being shaken by hand. The concentration of *Trichoderma pseudokoningii* spores in suspension was about 1 × 10^7 to 5 × 10^7 spores per ml. The suspension was then used as the inoculum to prepare fermented wheat bran (FWB). Wheat bran was chosen as the feed substrate for solid-state fermentation (SSF) and procured from the Formosa Oilseed Processing Corporation, Taichung, Taiwan.

**Solid-state fermentation (SSF)**

The techniques for large-scale production of FWB are similar to SSF in the lab. Thirty grams of wheat bran were first weighed into a sterilised bag before being mixed with enough deionised water to reach a moisture content of 50%. The contents of each bag were thoroughly mixed before being autoclaved at 121 ± 1 °C for 30 min. While waiting for the bags to cool, the autoclaved wheat bran was inoculated with 20 ml of *Trichoderma pseudokoningii* spore suspension per kg of wheat bran. The bags were then placed in an environmentally controlled room that was maintained at a temperature of 25 °C. To obtain fermentation samples, each bag was removed at regular 24 h intervals for assaying. All samples were then dried at 40 °C for 2 d. Finally, the dried products were ground in a mill, packed in plastic bags, sealed and stored at ambient temperature.

**Enzyme extraction and assays**

Each fermented substrate (3 g) was subjected to 10-fold dilution with deionised water and stirred for 30 min to obtain the extract solution. The solution was centrifuged at 3000 rpm at 4 °C for 10 min, and then filtered using Whatman No.1 filter paper before being analysed for cellulase and xylanase activities. Both enzymes were assayed by measuring the reducing sugars using the dinitrosalicylic acid (DNS) method (Miller 1959). The developed colours were measured at 540 nm. One international unit (IU) of cellulase and xylanase activity was defined as the quantity of enzyme required to liberate 1 μm of reducing sugar (glucose/xylose) of crude filtrate per minute under standard assay conditions (50 °C, pH 4.8 for cellulase and 55 °C, pH 5.3 for xylanase).

**Determination of the reducing sugar content**

Three grams of two substrates fermented for 0 and 4 d were subjected to 10-fold dilution with deionised water and stirred for 30 min to obtain the extract solutions. The solvent (deionised water) extracts were centrifuged at 3000 rpm for 10 min then filtered through Whatman No. 1 filter paper. The reducing sugar content of each sample was measured based on the procedures described by Miller et al. (1959), with minor modifications. Briefly, an aliquot of 1 ml extract solution was mixed with 1 ml dinitrosalicylic acid reagent and 1 ml deionised water, and then allowed to react for 5 min at 100 °C before being compared to a glucose solution standard. Absorbance was measured at 540 nm. The results were expressed as mg glucose equivalence/g extracts.

**In vitro adherence assay**

*Trichoderma pseudokoningii* was tested for its ability to adhere to the epithelial cells of the chicken crop. Epithelial cells were brushed from the crop of a 35 d old broiler. The cells were suspended in phosphate-
buffered saline (pH 7.3) at a density of \( \sim 10^4 \) to \( 10^5 \) cells per ml. *Trichoderma* from the potato dextrose agar (PDA) was washed and suspended in phosphate-buffered saline at a density of about \( 1 \times 10^7 \) cells per ml. 0.5 ml of the epithelial cell suspension was mixed with 0.5 ml of the *Trichoderma* suspension and incubated at 37°C, with rotation, for 30 min. Samples were then stained with crystal violet and observed by phase-contrast microscopy.

**Experimental birds and housing**

The experimental protocol was approved by the Animal Care and Use Committee of National Chung Hsing University, Taiwan. One hundred and eighty 1-day-old commercial Ross 308 male broiler chicks were randomly allocated to one of three dietary treatments, with three replicates and 20 birds per pen (total of 60 birds/treatment). At the beginning of the feeding trial, the average body weight of the birds was similar for all pens (\(-43.3\)–\(45.5\) g/bird). All chicks were wing-banded immediately after hatching and raised in a temperature controlled house. The birds were kept in floor pens 2.5 m wide and 4.00 m long, each containing a wire floor and rice husk litter material. From day 1–21, there were two fountain drinkers and one feed tray per pen. In the grower phase, fountain drinkers were replaced with nipple drinkers and the feed trays replaced with a feeder. Vaccines for Marek’s disease, Newcastle disease and infectious bronchitis were provided to chicks on their first day. Moreover, vaccines for Newcastle disease and infectious bursal disease were administered on day 14. Room temperature was maintained at 34 ± 1°C for the first 7 d, and then gradually decreased to 26 ± 1°C until the birds reached 21 d of age. A temperature of 26°C was maintained until the end of the 35 d.

**Feeding schedule and dietary composition**

The experiment was conducted for 35 d and there were 2 phases: a starter phase (1–21 d) and a grower phase (22–35 d). The broilers were allowed free access to water and feed. The dietary nutrient levels for both phases met National Research Council (1994) recommendations. The birds in the control group were fed corn-soybean meal (basal diet), the 10% WB group was fed the basal diet but with 10% replaced with WB and the 10% FWB group was fed the basal diet where 10% was replaced with FWB. Starter and grower diets were offered to the birds from 1 to 21 d and from 22 to 35 d of age, respectively. Neither anti-coccidial nor anti-bacterial supplements were added to the feed mixtures. The starter diet was formulated to have a metabolisable energy (ME) level and protein composition of 3050 kcal/kg ME and 23.0%, respectively, while the grower diet was 3175 kcal/kg ME and 21.0%, respectively (Table 1).

**Performance, serum and intestinal content collection**

The body weight of each broiler and feed intake value were measured in each pen at 1, 21 and 35 d of age. Body weight gains and feed conversion ratios (FCR) were calculated from this data. At 35 d, four birds were randomly selected from each pen for sampling. The birds were bled via the brachial vein, and 5 mL blood samples were collected from the wing vein. Samples were centrifuged at 2000×g for 15 min and the serum was stored at –20°C until being evaluated for total protein (T-P), albumin (ALB), serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels by a biochemical analyser kit (Technican RA-XT, NY) using an automatic biochemical analyser (RA-1000, Bayer Corp., NY). After the blood samples were collected, the chickens were euthanised by exsanguination and their abdominal cavities were opened. For each treatment group, the livers, hearts, bursas, leg muscles and contents of the ilea and caeca of three birds were collected for study.

**Microbial populations in ileal and caecal contents**

Fresh digestive samples were collected from the ileum and caecum. Samples were serially diluted in a phosphate-buffered saline solution for enumeration of microbial populations. Strains of *Clostridium perfringens* and *Coliform* were cultured with the reinforced clostridial agar (BD Difco TM) medium. All results for the microbial populations were repeated and the averaged values were used for statistical analysis. After anaerobic incubation at 37°C for 48 h, the microflora numbers were calculated. Bacterial populations were expressed as base-10 logarithm colony-forming units (CFU) per gram of intestinal contents.

**Caecal and ileal volatile fatty acid (VFA) determination**

Digestive samples were collected from the caecum to determine VFA levels, including acetate, propionate, butyrate and heptanoic acid. Approximately 1 g of the caecal contents was suspended in 5 mL of distilled water in a screw-capped tube. After samples were homogenised using an ultra-turrax, they were...
centrifuged for 10 min at 10,844 × g and 4 °C. After centrifugation, 1 mL of supernatant was transferred into an ampulla and 0.2 mL metapthosphoric acid solution was added. After the samples were homogenised, the ampullas were cooled in an ice bath for >30 min. The samples were then centrifuged for 10 min at 10,844 × g before analysis by GC. The GC was equipped with a flame ionisation detector (FID) and a polyethylene glycol column. The column was operated from 100 to 150 °C with highly purified N2 as the carrier gas, at 1.8 mL/min (Zhang et al. 2003). Volatile acid standard mixes (SUPELCO) were used as standard solutions.

Villus height and crypt depth

During the autopsy, the gastrointestinal tract was removed from the jejunum and ileum at the following junctures: (1) between the point of entry of the bile ducts and Meckel’s diverticulum and (2) between Meckel’s diverticulum to the ileo-caecal junction. The intestinal tracts were flushed with PBS and fixed in 10% formalin. Each sample used paraffin embedding procedures and was then stained with haematoxylin and eosin. The prepared slides were observed with a light microscope. Villus height was from the tip of the villus to the villus-crypt junction; crypt depth was the depth of the invagination between adjacent villi. Morphological indices were measured using the Motic Image Plus 2.0 analysis system (Motic Instruments, Richmond, Canada).

| Ingredient, g/kg DM | Starter diet (1–21 days) | Finisher diet (22–35 days) |
|---------------------|--------------------------|---------------------------|
|                     | Control | WB, 10% | FWB, 10% | Control | WB, 10% | FWB, 10% |
| Corn, yellow         | 474.4   | 338.9   | 350.7   | 520.5   | 385.1   | 397.08  |
| Soybean meal (CP 44%)| 478.5   | 454     | 444     | 433     | 409     | 398     |
| WB                  | 0       | 100     | 0       | 0       | 100     | 0       |
| FWB                | 0       | 0       | 100     | 0       | 0       | 100     |
| Soybean oil         | 1.1     | 58.4    | 56.2    | 6.5     | 63.9    | 62.0    |
| Monocalcium phosphate | 18.2  | 18.8    | 18.8    | 16.2    | 16.3    | 16.8    |
| Calcium carbonate   | 16      | 16      | 16      | 13.3    | 13.3    | 13.3    |
| L-Lysine-HCl        | 1.6     | 2.8     | 3.1     | 0.9     | 2.06    | 2.38    |
| DL-Methionine       | 3.7     | 4.5     | 4.6     | 3.10    | 3.84    | 3.94    |
| NaCl                | 3.7     | 3.8     | 3.8     | 3.7     | 3.7     | 3.7     |
| Choline-Cl          | 0.8     | 0.8     | 0.8     | 0.8     | 0.8     | 0.8     |
| Vitamin premix a    | 1       | 1       | 1       | 1       | 1       | 1       |
| Mineral premix b    | 1       | 1       | 1       | 1       | 1       | 1       |
| Total               | 1000    | 1000    | 1000    | 1000    | 1000    | 1000    |

Calculated nutrient value

|                | ME, kcal/kg DM | Crude protein, %DM | Calcium, %DM | Total Phosphorus, %DM | Available Phosphorus, %DM | Lysine, %DM | Methionine + Cystein, %DM |
|----------------|----------------|--------------------|--------------|-----------------------|--------------------------|-------------|---------------------------|
| Control        | 3050.8         | 23                 | 1.05         | 0.77                  | 0.5                      | 1.43        | 1.07                       |
| WB, 10%        | 3050.1         | 23                 | 1.05         | 0.74                  | 0.5                      | 1.43        | 1.07                       |
| FWB, 10%       | 3050.1         | 23                 | 1.05         | 0.73                  | 0.5                      | 1.43        | 1.07                       |
| Control        | 3175           | 21                 | 0.9          | 0.71                  | 0.45                     | 1.25        | 0.96                       |
| WB, 10%        | 3175.02        | 21                 | 0.9          | 0.68                  | 0.45                     | 1.25        | 0.96                       |
| FWB, 10%       | 3175.02        | 21                 | 0.9          | 0.67                  | 0.45                     | 1.25        | 0.96                       |

Analysed nutrition value

|                | GE, kcak/kg DM | Crude protein, %DM | NDF, %DM | ADF, %DM | Ash, %DM |
|----------------|----------------|--------------------|----------|----------|----------|
| Control        | 4193           | 23.1               | 58.9     | 13.2     | 6.8      |
| WB, 10%        | 4305           | 23.5               | 51.8     | 15.3     | 6.7      |
| FWB, 10%       | 4488           | 22.9               | 51.0     | 14.9     | 7.1      |
| Control        | 4188           | 22.2               | 58.6     | 13.2     | 6.4      |
| WB, 10%        | 4483           | 21.2               | 55.3     | 15.3     | 6.1      |
| FWB, 10%       | 4476           | 21.9               | 46.2     | 14.4     | 6.9      |

WB: wheat bran; FWB: fermented wheat bran.

aSupplied per kg of diet: Vit A 15,000 U; Vit. D3 3000 U; Vit. E 30 mg; Vit. K3 4 mg; Riboflavin 8 mg; Pyridoxine 5 mg; Vit. B12 25 µg; Ca-pantothenate 19 mg; Niacin 50 mg; Folic acid 1.5 mg; Biotin 60 µg.

bSupplied per kg of diet: Co (CoCO 3) 0.255 mg; Cu (CuSO4·5H2O) 10.8 mg; Fe (FeSO4·H2O) 90 mg; Zn (ZnO) 68.4 mg; Mn (MnSO4·H2O) 90 mg; Se (Na2SeO3) 0.18 mg.

**Morphology and incidence of non-specific pathological lesions**

At the end of the experiment (d 35), one bird per replicate cage from each treatment group (total of 3 birds/treatment) was randomly selected and euthanised. During the autopsy, the livers, hearts, bursa and leg muscles were removed. Visual observed of carcase and the macroscopic anatomical dissection of the gastrointestinal tract as well as the abdominal organ, and then fixed in 10% buffered formalin. Each sample used paraffin embedding procedures and was then stained with haematoxylin and eosin. The prepared slides were observed with a light microscope. Prepared slides were observed with a light microscope. Prepared slides were observed with a light microscope.
slices were examined by a veterinarian for histopathological evaluation. Severity of lesions was graded according to the criteria described by Shackelford et al. (2002). Degree of lesions was graded from one to five depending on severity: 1 = minimal (< 1%), 2 = slight (1–25%), 3 = moderate (26–50%), 4 = moderate/severe (51–75%) and 5 = severe/high (76–100%).

Statistical analysis

Data was subjected to ANOVAs as a completely randomised design using the GLM function of the SAS software (SAS Institute 2004). Determination of the significant statistical differences among the mean values of the three treatment groups used Tukey’s honestly significant difference test, with a significance level of \( p < .05 \).

Results

Cellulase and xylanase activity

Cellulase and xylanase activity of WB during SSF with Trichoderma pseudokoningii is shown in Table 2. As SSF progressed, cellulase and xylanase activity in the substrate increased before peaking after 4 d. The cellulase and xylanase activity of fermented WB on Day 4 were 15.19 U/g and 179.7 U/g, respectively.

Reducing sugar content

The reducing sugar content of WB during SSF with Trichoderma pseudokoningii is presented in Table 2. The reducing sugar content of WB increased from 14.71 mg/g to 21.1 mg/g after solid-state fermentation with Trichoderma pseudokoningii.

In vitro adhesion to epithelial cells

The mycelium of Trichoderma pseudokoningii is filamentous while the spores of Trichoderma are spherical (Figure 1A and 1B). Therefore, it was determined that Trichoderma might adhere to the epithelial cells of the chicken crop (Figure 1D) compared to without adhered cells by Trichoderma spore (Figure 1C).

Table 2. Analysis of the active ingredients and enzyme activity of fermented wheat bran by Trichoderma pseudokoningii.

| Item                      | Fermented days |
|---------------------------|----------------|
|                           | 0              | 4               |
| Cellulase activity, U/g   | 0.79 ± 0.07    | 15.19 ± 0.15    |
| Xylanase activity, U/g    | 0.28 ± 0.04    | 179.7 ± 6.62    |
| Reducing sugar, mg/g      | 14.71 ± 0.46   | 21.1 ± 0.46     |

The result is expressed as the mean ± standard deviation (\( n = 3 \)).

Figure 1. Photomicrography of Trichoderma adhering to crop epithelial cells of chicken crop in vitro. (A) The mycelium of Trichoderma pseudokoningii. (B) The spore of Trichoderma pseudokoningii. (C) The epithelial cell of crop. (D) The crop epithelial attached spore of Trichoderma pseudokoningii.
**Growth performance**

The effects of dietary supplementation with FWB on the growth performance of 1- to 35-d-old broilers are shown in Table 3. There were no significant differences in body weight or weight gain among all treatment groups from 1 to 21 d of age. However, the 10% FWB treatment group had significantly lower feed consumption than the control group. Among all treatment groups, there were no significant differences for each growth parameter from 22 to 35 d of age. Overall, the 10% FWB treatment group had lower feed consumption, resulting in an improved FCR compared to the control group (p < .05).

**Microbial population in ileum and caeca**

The effects of dietary supplementation with FWB on the microbial population in broiler ilea and caeca after Table 3. Effect of fermented wheat bran (FWB) supplemented in diet on growth performance of 1–35 d-old broilers.

| Item                       | Control  | 10% WB  | 10% FWB | SEM | p value |
|----------------------------|----------|---------|---------|-----|---------|
| 1–21 days                  |          |         |         |     |         |
| Body weight, g'            | 743      | 725     | 734     | 8.06| .352    |
| Feed consumption, g'       | 962b     | 913ab   | 808b    | 31.5| .035    |
| Weight gain, g'            | 661      | 647     | 662     | 12.5| .644    |
| FCR                       | 1.45a    | 1.41ab  | 1.22b   | 0.06| .056    |
| 22–35 days                 |          |         |         |     |         |
| Body weight, g             | 2130     | 2043    | 2092    | 25.1| .122    |
| Feed consumption, g        | 2036     | 1957    | 1925    | 60.2| .453    |
| Weight gain, g             | 1387     | 1318    | 1358    | 29.4| .314    |
| FCR                       | 1.47     | 1.49    | 1.42    | 0.04| .478    |
| 1–35 days                  |          |         |         |     |         |
| Feed consumption, g        | 2998ab   | 2871ab  | 2734b   | 64.7| .073    |
| Weight gain, g             | 2049     | 1965    | 2021    | 29.3| .201    |
| FCR                       | 1.44ab   | 1.45a   | 1.35b   | 0.01| .003    |

SEM: standard error of the mean; WB: wheat bran; FWB: fermented wheat bran; FCR: feed conversion ratio.

a,bMeans within the same rows without the same superscript letter are significantly different (p < .05).

The results are provided as the means of sixty birds in each control and treatment group (n = 60).

The results are provided as the means of three replicates (20 birds/rePLICATE) in each control and treatment group (n = 3).

Table 4. Effect of fermented wheat bran (FWB) supplemented in diet on Coliform and Clostridium perfringens count in intestinal content of 35 d old broilers.

| Item                                 | Control  | 10% WB  | 10% FWB | SEM | p value |
|--------------------------------------|----------|---------|---------|-----|---------|
| Coliform, Log10 cfu/g                |          |         |         |     |         |
| Ileum                                | 8.21     | 6.68b   | 7.61ab  | 0.30| .054    |
| Caecum                               | 9.31     | 8.89    | 8.98    | 0.13| .135    |
| Clostridium perfringens, Log10 cfu/g |          |         |         |     |         |
| Ileum                                | 8.25     | 8.33    | 7.99    | 0.13| .281    |
| Caecum                               | 9.57     | 9.41    | 9.51    | 0.11| .607    |

WB: wheat bran; FWB: fermented wheat bran; SEM: standard error of the mean.

a,bMeans within the same rows without the same superscript letter are significantly different (p < .05).

The results are provided as the means of three replicates (4 birds/rePLICATE) in each control and treatment group (n = 3).

35 d are presented in Table 4. There were no significant differences in the caecal bacterial population among all treatment groups. The Coliform bacteria count in the ileum was lower in the 10% WB group than the control, however there were no differences between the 10% WB and 10% FWB treatment groups in the C. perfringens count.

**Volatile fatty acids (VFA) in caeca**

Table 5 shows the effects of dietary supplementation with FWB on the VFA concentration in the caeca of broilers. There were no significant differences in acetic acid and butyric acid levels among all treatment groups, however, the 10% FWB group had significantly less propionic acid compared to the control (p < .05). There was a significant difference in heptanoic acid between the 10% FWB and 10% WB groups.

**Intestinal morphology**

Table 6 shows the effects of dietary supplementation with FWB on the intestinal morphology of broilers after 35 d. Chickens in the 10% FWB treatment group had greater ileal villus height than both the control and 10% WB groups.

Table 5. Effect of fermented wheat bran (FWB) supplemented in diet on caecal VFA concentration of broilers (35 d)e.

| Item                                 | Control  | 10% WB  | 10% FWB | SEM | p value |
|--------------------------------------|----------|---------|---------|-----|---------|
| Acetic acid, µmole/g                 |          |         |         |     |         |
| 10% FWB                              | 13.99    | 15.68   | 12.73   | 0.93| .241    |
| Propionic acid, µmole/g              |          |         |         |     |         |
| 10% WB                               | 3.48     | 2.60    | 2.25    | 0.32| .085    |
| Butyric acid, µmole/g                |          |         |         |     |         |
| 10% WB                               | 2.80     | 2.82    | 2.41    | 0.21| .047    |
| Heptanoic acid, µmole/g              |          |         |         |     |         |
| 10% WB                               | 0.017ab  | 0.038a  | 0.013ab | 0.003| .039   |

a,bMeans within the same rows without the same superscript letter are significantly different (p < .05).

cThe results are provided as the means of three replicates (4 birds/rePLICATE) in each control and treatment group (n = 3).

dVFA: Volatile fatty acid; WB: wheat bran; FWB: fermented wheat bran.

dThe results are provided as the means of twenty spots corresponding to three birds for the control group (corn-soybean meal diet), 10% WB and 10% FWB.

Table 6. Effect of fermented wheat bran (FWB) supplemented in diet on intestinal morphology of 35 days old broilers.

| Item                                  | Control  | 10% WB  | 10% FWB | SEM | p value |
|---------------------------------------|----------|---------|---------|-----|---------|
| Jejunum                                |          |         |         |     |         |
| Villus height, µm                      |          |         |         |     |         |
| Control                               | 1162     | 1192    | 1167    | 19.41| .629    |
| 10% WB                                | 1373     | 1342    | 1357    | 5.12<.0001|
| 10% FWB                               | 8.52     | 8.37b   | 8.37a   | 0.31<.0001|
| Crypt depth, µm                       |          |         |         |     |         |
| Control                               | 163      | 149     | 161     | 4.19| .157    |
| 10% WB                                | 1615     | 149     | 161     | 4.19| .157    |
| 10% FWB                               | 6.98     | 6.98ab  | 6.98a   | 0.16<.0001|

WB: wheat bran; FWB: fermented wheat bran; SEM: standard error of the mean.

a,bMeans within the same rows without the same superscript letter are significantly different (p < .05).

cThe results are provided as the means of twenty spots corresponding to three birds for the control group (corn-soybean meal diet), 10% WB and 10% FWB.
and 10% WB treatment groups. There were no significant differences in ileal crypt depth among all treatments. The 10% FWB group had a higher villus height to crypt depth ratio in the ileum compared to the control (p < .05). There were no significant differences in the jejunal villus height among all treatment groups, however, the crypt depth in the 10% FWB treatment group was significantly lower than the control (p < .05). As a result, the 10% FWB group had a significantly greater villus height to crypt depth ratio than the control in the jejunum (p < .05). Photomicrography of the jejunum and ileum of 35-day-old broilers fed with control and fermented wheat bran is presented in Figure 2.

**Serum characteristics**

The effects of dietary supplementation with FWB on serum characteristics at 35 d of age are presented in Table 7. There were no significant differences in serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), total protein (T-P) or albumin (ALB) levels among all treatment groups.

| Item          | Control | WB    | FWB   | SEM   | p value |
|---------------|---------|-------|-------|-------|---------|
| U/l           | SGOT    | 221.7 | 251.3 | 285.0 | 28.68   | .45     |
|               | SGPT    | 4.0   | 4.8   | 4.5   | 0.4     | .44     |
| g/dl          | T-P     | 3.08  | 3.08  | 3.18  | 0.07    | .54     |
|               | ALB     | 0.93  | 0.97  | 0.80  | 0.04    | .08     |

*aEach value represents the mean of four replicates.

WB: wheat bran; FWB: fermented wheat bran; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic-pyruvic transaminase; T-P: total protein; ALB: albumin.

The results showed there were no morphological changes or incidences of non-specific pathological lesions in the 10% FWB group when compared to the other groups.

**Evaluation of FBW meal economic benefit**

An evaluation of the economic benefits of adding FBW meal to the broiler diet is summarised in Table 8. The income over feed cost (IOFC) of the control and FBW meal groups were 70.4 and 60.2 NT$/bird, respectively.

**Discussion**

Zijlstra et al. (2010) reported that supplementation with NSP-degrading enzymes (such as cellulase and xylanase) can be useful in increasing the digestive
utilisation of nutrients in NSP-rich foodstuffs. NDF and ADF are significantly reduced in rice husks fermented with 
*Trichoderma viride* (Aderolu et al. 2007). Omwango et al. (2013) reported that pineapple waste fermented with 
*Trichoderma viride* significantly increased crude protein levels, but decreased the amount of crude fibre during SSF. Ezekie and Aworh (2013) also showed that solid state fermentation of cassava peel with *Trichoderma viride* significantly increased crude protein while crude fibre was reduced. During the SSF process, cellulase and xylanase activity in fermented products increases, effectively degrading lignocellulosic biomass with reducing sugars.

Our study indicated that cellulase and xylanase produced by the fermentation of wheat bran with *Trichoderma pseudokoningii* partially eliminated the NDF (55.9 to 50.3%) and ADF (22.6 to 21.0%). The crude protein level increased from 19.0 to 22.4%; possibly due to fungi growing on the substrates, further utilising available nutrients, even though the DM level showed no significant difference between the groups (FWB: 94.0% vs. WB: 93.0%). In theory, fungi synthesise and excrete numerous cellulolytic enzymes that break down the non-starch polysaccharides into simple sugars that enter the fungi to promote biosynthesis and metabolic activities. Once the bioconversion of sugars to single-cell proteins or microbial proteins occurs, the crude protein is further increased after fermentation (Raimbault 1998).

In a previous study, Ng et al. (2002) show that compared to non-fermented products, *Trichoderma koningii*-fermented palm kernel meal (FPKM) could increase the reducing sugar and crude protein levels. When FPKM was incorporated into their diet, red hybrid tilapia demonstrated poorer growth performance than those fed untreated PKM. The authors suggested that some interference with unknown anti-nutrients or an amino acid deficiency from fungal proteins restricted the amount of protein available to the fish (Dabrowski et al. 1980; Ng et al. 2002). In another study, *Aspergillus niger* fermented *Terminalia catappa* fruit meal replaced dietary maize at 0, 20, 40, 60 or 80% and was fed to broilers; there were no significant differences in weight gain and FCR between the control

Table 8. Evaluation of the economic benefit of fermented wheat bran (FWB) supplemented in diet.

| Item                        | Control | 10% WB | 10% FWB |
|-----------------------------|---------|--------|---------|
| Feed cost, NT$/bird         |         |        |         |
| 1–35 days                   | 24.7    | 34.5   | 33.2    |
| Meat income, NT$/bird       |         |        |         |
| 1–35 days                   | 95.1    | 91.3   | 93.4    |
| Income over feed cost, NT$/bird |       |        |         |
| 1–35 days                   | 70.4    | 56.7   | 60.2    |

Feed cost: Basing on the costs (NT$/kg) of the ingredients as follows: soybean meal 14.27, fish meal 39.66, wheat bran 7.30, fermented wheat bran 10.0, Soybean oil 50.0, monocalcium phosphate 16.95, Calcium carbonate 1.94, L-Lysine-HCl 300, DL-Methionine 230, salt (HCl) 2.70, choline chloride, 50% 33.80, vitamin premix 135.00, and mineral premix 39.00. The fees for processing of basal ration per kg were 8.76 for grain mixture of control group, 12.5 for 10% WB group and 12.7 for 10% FWB during 1–21 day, respectively. The fees for processing of basal ration per kg were 8.00 for grain mixture of control group, 11.8 for 10% WB group and 11.9 for 10% FWB during 22–35 day, respectively.

Figure 3. The pathological section of hepatic tissue, myocardial tissue, bursal tissue and leg muscle tissue of 35 days old broiler of control (A, D, G, J), wheat bran (B, E, H, K) and fermented wheat bran (C, F, I, L) groups, respectively.
and the 40% replaced treatment group. This indicates that replacing 40% of the diet was enough to support growth and feed conversion (Apata 2010). In our study, the 10% FWB group had lower feed consumption than the other groups; moreover, we found a lower FCR in the 10% FWB group compared to the control. As previously mentioned, fungi excreted exogenous enzymes after solid-state fermentation; it broke down lingo-cellulolytic bonds to increase the amount of soluble carbohydrates. This could provide a positive effect on the growth performance of broilers. Intestinal microflora influence intestinal health, suppression of pathogens and growth promotion in the host (Niba et al. 2009; Sun et al. 2013). Nian et al. (2011) reported that wheat-based diets supplemented with exogenous xylanase decreased the number of coliform bacteria in the ilea of broilers. In the current study, compared to the control group, the number of coliform bacteria was decreased in the ilea and caeca of broilers supplemented with 10% FWB; similarly, the number of Clostridium perfringens was decreased in the ileum.

Hindgut fermentation in broilers results in the breakdown of dietary fibre into simpler sugars, possibly yielding more VFA than large molecular carbohydrates (Marounek et al. 1999). Wheat-based diets supplemented with different amounts of enzymes (e.g. xylanase, cellulose, β-glucanase) increased the level of acetic acid in the caeca of 21-day-old broilers, while the levels of propionic and butyric acid decreased (Wang et al. 2005). Sharmila et al. (2014) reported that there was no significant difference in the percentage of acetic and propionic acid in the caeca of 35-day-old broilers fed palm kernel meal-based diet supplemented with cellulase or xylanase. In the current study, the caecal of acetate, propionate and butyrate levels were no different among the groups. These results are similar to those of Urlings et al. (1993) and Fransen et al. (1995). They found a higher pH in the faeces of pigs given fermented feed compared to those given normal feed. This increase in pH was related to a lower production of VFAs. According to the above results, VFAs produced through caecal fermentation can be quite variable as they may also be influenced by non-carbohydrate compounds such as protein, lignin, and fatty acids. Besides the degradation of high-fibre feeds, the caecum also plays an important role in the hydrolysis of nitrogenous compounds such as proteins and amino acids to generate ammonia, amines, phenol and branched fatty acid chains (Sharmila et al. 2014).

A larger villus height increases the surface area, allowing for greater absorption of available nutrients and further improving intestinal health (Baurhoo et al. 2007). Furthermore, the crypt can be thought of as the villus factory; a large crypt suggests faster tissue turnover and more energy demands for histogenesis (Awad et al. 2009). Xu et al. (2012) reported the effects on the intestinal morphology of broilers when 10% of the soybean meal (SBM) was replaced with Lactobacillus fermentum and Bacillus subtilis co-fermented rapeseed meal (RSM). Significant increases in villus height and villus height to crypt depth ratio in the jejunum were observed, but there were no significant differences in the ileum. A previous study demonstrated increased villus height and decreased crypt depth in the jejunal mucosa of broilers fed Aspergillus oryzae 3.042 fermented soybean meal (FSBM), but there were no significant effects on the ileal morphology (Feng et al. 2007). In the current study, there were no significant differences in the jejunal villus height among all treatment groups; on the other hand, jejunal crypt depth was decreased in the 10% FWB group, compared to the control. In addition, the ileal villus height was significantly greater in the 10% FWB group compared to the control. There were no significant differences in ileal crypt depth among the treatment groups. The improvement in intestinal morphology may be mostly due to the decrease of anti-nutritional factors and the degradation of structural polysaccharides.

Blood chemical parameters are important indicators of the physiology and health of livestock (Etim et al. 2013). Serum chemical parameters, such as SGOT, SGPT, T-P and ALB are mainly evaluated for liver and kidney function. In a previous study, replacing maize with Terminalia catappa fruit meal (FTCM) fermented with Aspergillus niger produced serum values for T-P and ALB in broilers that were similar to the control, right up until 40% of the maize had been replaced by FTCM (Apata 2010). Similarly, there were no significant differences in SGOT, SGPT, T-P or ALB levels. Therefore, in the current study, it may be presumed that the Trichoderma-treated diet had no negative effects on the liver function of the broilers.

Some probiotic species possess adhesive capabilities to avoid the enterogastric peristalsis of intestinal epithelial cells. Lactobacillus species possess adhesive capabilities, hindering pathogens from invading and adhering to the host cells of the intestinal tract (Bernet et al. 1993, 1994; Coconnier et al. 1993a,b). In the current study, it was determined that Trichoderma pseudokoningii might adhere to the epithelial cells of the chicken crop. This mechanism will be investigated in the near future.
Conclusions

The SSF of wheat bran with *Trichoderma pseudokonigii* raised the levels of reducing sugars. Therefore, replacing 10% of the diet with fermented wheat bran could not only improve the growth performance of broilers, but also provide optimal intestinal morphology without any adverse effects on blood chemical parameters.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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