Effects of solid-state fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on growth performance and intestinal microbiota in broiler chickens

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ABSTRACT

This study evaluated the effects of 10% solid-state fermentation (SSF) wheat bran (WB) by *Bacillus amyloliquefaciens* (BA) and *Saccharomyces cerevisiae* (SC) in broiler diets, on growth performance, intestinal morphology, and intestinal microbiota and serum characteristics of broiler chickens. The results showed that fermented wheat bran with BA or BA and SC reduced NDF and ADF contents in WB material. A total of 240 male broilers (Ross 308) were randomly allocated into four feeding groups: (1). Basal diet with 10% WB (control treatment). (2). Basal diet that replaced WB with fermented WB by BA (FWBA). (3). Fermented WB by SC (FWSC), and (4). Fermented WB by BA and SC (FWBA + SC), each group was fed these particular diets for 35 d. Results demonstrated that replacement of WB with FWBA and FWBA + SC improved feed conversion ratio (d 1 to d 35) compared to the control group. Moreover, the 10% FWBA group significantly enhanced the lactic acid bacteria count in the ileum of 35 d old broilers. Compared to the control group, the 10% FWBA and 10% FWSC groups, showed significantly increased ileal lactic acid levels, and in the 10% FWSC group, the ileum villus height was significantly increased in 35 d old broilers. However, treatments with 10% FWBA and 10% FWBA + SC had a tendency to reduce serum cholesterol. In conclusion, a 10% FWBA replacement in the diet could ameliorate health status in broilers by improving growth performance, modulating intestinal microbiota and increasing lactic acid in the ileum.

Introduction

Techniques including direct-fed microbials (DFM) and microbial fermentation have been applied to improve growth performance and enhance the health status of poultry. The microorganisms, including *Lactobacillus*, *Bacillus* and *Saccharomyces cerevisiae* (SC), have been studied as potential feed additives due to their outstanding production of extracellular enzymes including protease, amylase, cellulase and lipase (Chen et al. 2009; Salim et al. 2013). These enzymes may increase the nutrient digestibility of proteins, carbohydrates and lipids in broilers (Salim et al. 2013). *Bacillus amyloliquefaciens* (BA), a member of the genus *Bacillus*, is a spore-producing gram-positive bacteria. BA can improve the growth performance and modulate the intestine microbiota of broilers (Ahmed et al. 2014). SC, one of the most widely commercialised types of yeast, has long been fed to animals, and it has a beneficial effect on growth, modulation of intestinal microbiota and pathogen inhibition in broilers (Mountzouris et al. 2015). In contrast, there were reports that showed that body weight gain (BWG) was not affected by the addition of dietary DFM in broiler diets (Lee et al. 2010). Because of the unstable effects of supplemented DFM in broiler diets, microbial fermentation of by-products may be a substituted way to enhance the benefit of microbial and improve nutrient availability of by-products (Chen et al. 2009).

Recent studies have reported using fungal inoculums for the fermentation of agricultural by-products in order to enhance their nutritional value (Sharma & Arora 2010). Solid-state fermentation (SSF) is the growth of organisms on moist substrates in the absence of free-flowing water. The use of SSF for
the production of enzymes and other products has many advantages over submerged fermentation. A very important advantage is that it permits the use of agricultural and agro-industrial residues as substrates which are then converted into products with high-commercial values like secondary metabolites (Reda et al. 2014). A large number of fungal species were known to grow well on moist substrates in the absence of free-flowing water. As a result, most studies involving SSF have been conducted using fungi. However, there are few reports of bacterial strains or mixtures of fungi and bacteria being used successfully for the improvement of by-product utilisation by using SSF in broilers.

Wheat bran (WB), as the main by-product of wheat flour processing, contains a high amount of total dietary fibre (451 g/kg), and other relevant compounds including protein (160 g/kg), fat (47 g/kg), available carbohydrates (177 g/kg) and minerals (61.5 g/kg) (Souci & Kirchhoff 2008). However, it is well known that increasing fibre content negatively correlates with animal feed intake, and affects growth performance. Therefore the use of wheat bran was limited in animal feeding. It is hypothesised that SSF may degrade fibres and eliminate the disadvantage of wheat bran in broiler diets.

The previous studies generally evaluated the effects of fermented complete feed on animals (Chen et al. 2009), but once composition of complete diets changes, fermenting condition should alter as well. Hence, different formulations of complete diets among animals and feeding periods limited the application of SSF. However, by fermenting a simple feed ingredient, like wheat bran, it could be formulated in different diets which remove the practical limitation from feed fermentation. There are few reports that have focussed on the use of fermented wheat bran in broiler diets. Therefore, this study is intended to demonstrate the effect of the replacement of 10% wheat bran in basal diets by solid-state fermented wheat bran with BA and SC on the growth performance, intestinal microbiota, serum characteristics and intestinal morphology in broiler chickens.

Materials and methods

Solid-state fermentation (SSF)

Wheat bran was mixed with 60% distilled water, and added to a 10% broth culture of BA, 10% broth culture of SC, and a mixture solution of with a 5% broth culture of BA and a 5% broth culture of SC, respectively. The BA and SC used in this study were screened and selected by our laboratory (Teng et al. 2017). The broth cultures were prepared by inoculating BA in Lysogeny broth (LB) and SC in Yeast-Mald (YM) at 37°C for 24 h and the colony-forming units was verified achieving 1 x 10^7 CFU/mL by plating serial 10-fold dilutions in duplicate into LB agar plates and YM agar plates. After fermentation at 30°C for 3 d, the fermented wheat brans were dried at 40°C for 4 d and mashed to formulate broiler diets.

Measurement of total dietary fibre

Total dietary fibre was evaluated using the Megazyme kit (K-TDFR 200A, Megazyme Inc., Chicago, IL). One gram sample was mixed with 50 mL phosphate buffer (pH 6.0), and samples were digested by 50 μL thermostable α-amylase in boiling water bath for 15 min, by 100 μL protease at 60°C for 30 min, and by 200 μL α-amylolucosidase at 60°C for 30 min, sequentially. After enzyme digestions, soluble dietary fibre was precipitated with 76% (v/v) ethanol. Dietary fibre residues were isolated by filtration through fritted crucibles and corrected by analysing ash and protein contents in the residues.

Radial enzyme diffusion assay

The enzyme activities of were assayed by radial enzyme diffusion method described by Walsh et al. (2005). After fermentation, 5 g fermented samples were mixed with 45 mL RO water to extract crude enzyme in ice bath for 10 min. Crude enzyme extractions were loaded to agar plates containing 1% casein, 0.3% xylan, and 0.1% carboxymethyl-cellulose for protease, xylanase, and cellulase radial diffusions, respectively. RO water was used as negative control group and commercial enzymes were used as positive control groups.

Feeding schedule and dietary composition

The experiment lasted for 35 d during which there were 2 phases: Starter (1 to 21 d) and finisher (22 to 35 d). The diets (in mash form) and water were provided ad libitum. The birds in the control group were fed a diet based on maize-soybean with 10% WB, and the other three groups were provided experimental diets based on the control group’s diet but the WB was replaced with fermented WB by BA (FWBA), with fermented WB by SC (FWSC) and fermented WB by BA and SC (FWBA + SC). Starter and finisher diets were provided to the birds from 1 to 21 d and from 22 to 35 d of age, respectively (Table 1). During the entire experimental period (35 d), the diets were formulated
to meet the requirements suggested by the Ross Broiler Management Manual (2009). The proximate composition was analysed according to the AOAC (1980) showed no major deviations from the calculated values. The feed mixtures contained no anticoccidial or antibacterial supplements. The method of NDF determination was following the procedure described by Van Soest et al. (1991).

### Experimental birds and housing

This study was conducted at National Chung Hsing University, Taiwan. The experimental protocol for animal use was approved by the Animal Care and Use Committee. A total of two hundred and forty male broilers (Ross 308) were randomly allocated to four treatments, each of which had three replicate pens per treatment and 20 birds per pen (total of 60 birds per treatment). Initial average body weight (BW) of the birds were similar among different pens (average 46.0 to 46.5 g/bird approximately). All of the birds were raised in the pens (2 × 4 m²) with rice husk as litter and housed in a temperature-controlled environment. The temperature was maintained at 33 ± 1 °C until the birds reached 7 d of age, and was gradually decreased to 27 ± 1 °C until the birds reached 21 d of age; after this point, the broilers were maintained at 27 ± 1 °C.

### Performance, serum, intestinal content, and faeces collection

The BW of chickens per pen and feed intake was recorded at 1, 21 and 35 d of age. BWG and feed conversion ratio (FCR) were calculated. On Day 35, 12 chickens per treatment (four birds from each pen) close to the average weight were selected for sampling of blood, intestinal content and faeces. Fifty grams of faeces per treatment, excreted over 2h, were collected randomly into three plastic plates twice (six replicates in total) to determine ammonia content. Blood from the brachial vein was collected (5 ml) via cardiac puncture using a vacutainer tube. The blood was centrifuged at 3000 × g for 30 min to obtain the serum, and was stored at −20 °C, then analysed for serum characteristics. After the blood was collected, the birds were euthanized by exsanguination and the ileal and excreta contents were collected in sterile plastic plates for subsequent study.

### Table 1. Ingredients and chemical composition of the experimental diets for broilers.

| Ingredient                                | Starter diet (1–21days) | Finisher diet (22–35days) |
|-------------------------------------------|-------------------------|---------------------------|
|                                           | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC |
| Maize, yellow                             | 338.9   | 337.7    | 341      | 337          | 385.1   | 384.9    | 387.4    | 384          |
| Soybean meal (CP 44%)                     | 154     | 155      | 152      | 155          | 83.8    | 83.8     | 81.6     | 84.4         |
| Full fat soybean meal                     | 300     | 300      | 300      | 300          | 325     | 325      | 325      | 325          |
| Wheat bran                                | 100     | 0        | 0        | 100          | 0       | 0        | 0        | 0            |
| FWBA                                      | 0       | 100      | 0        | 0            | 0       | 100      | 0        | 0            |
| FWSC                                      | 0       | 0        | 100      | 0            | 0       | 0        | 100      | 0            |
| FSBA + SC                                 | 0       | 0        | 0        | 100          | 0       | 0        | 0        | 100          |
| Soybean oil                               | 58.4    | 58.6     | 58       | 58.6         | 63.9    | 63.9     | 63.5     | 64.1         |
| Monocalcium phosphate                     | 18.8    | 18.8     | 18.8     | 18.8         | 16.7    | 16.7     | 16.7     | 16.7         |
| Calcium carbonate                         | 16      | 16       | 16       | 16           | 13.3    | 13.3     | 13.3     | 13.3         |
| L-Lysine-HCl                              | 2.8     | 2.8      | 2.9      | 2.8          | 2.1     | 2.1      | 2.1      | 2.0          |
| DL-Methionine                             | 4.5     | 4.5      | 4.5      | 4.5          | 3.8     | 3.8      | 3.8      | 3.8          |
| NaCl                                      | 3.8     | 3.8      | 3.8      | 3.8          | 3.7     | 3.7      | 3.7      | 3.7          |
| Choline-Cl                                | 0.8     | 0.8      | 0.8      | 0.8          | 0.8     | 0.8      | 0.8      | 0.8          |
| Vitamin premixa                           | 1       | 1        | 1        | 1            | 1       | 1        | 1        | 1            |
| Mineral premixb                           | 1       | 1        | 1        | 1            | 1       | 1        | 1        | 1            |
| Total                                     | 1000    | 1000     | 1000     | 1000         | 1000    | 1000     | 1000     | 1000         |
| Calculated nutrient value                 |         |          |          |              |         |          |          |              |
| ME, MJ/kg                                 | 12.8    | 12.8     | 12.8     | 12.8         | 14.0    | 14.0     | 14.0     | 14.0         |
| Crude protein, %                          | 23.0    | 23.0     | 23.0     | 23.0         | 21.0    | 21.0     | 21.0     | 21.0         |
| Calcium, %                                | 1.05    | 1.05     | 1.05     | 1.05         | 0.90    | 0.90     | 0.90     | 0.90         |
| Total Phosphorus, %                       | 0.75    | 0.76     | 0.75     | 0.76         | 0.68    | 0.68     | 0.68     | 0.68         |
| Available Phosphorus, %                   | 0.50    | 0.51     | 0.50     | 0.51         | 0.45    | 0.45     | 0.45     | 0.45         |
| Lysine, %                                 | 1.43    | 1.43     | 1.43     | 1.43         | 1.25    | 1.25     | 1.25     | 1.25         |
| Methionine + Cystein, %                   | 1.07    | 1.07     | 1.07     | 1.07         | 0.96    | 0.96     | 0.96     | 0.96         |
| FWBA: Fermented wheat brans by Bacillus amyloliquefaciens; FWSC: Fermented wheat brans by Saccharomyces cerevisiae; FWBA + SC: Fermented wheat brans by Bacillus amyloliquefaciens and Saccharomyces cerevisiae.

*Supplied per kg of diet: Vit A 15000 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 (CoCO₃) 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.
**Ileal microbial populations**

One gram of the ileal sample was serially diluted with 9 mL of 0.9% sterile saline (1:10 dilution) and mixed thoroughly. Viable counts of bacteria in the ileal samples were then conducted by plating serial 10-fold dilutions in duplicate into TSC agar (tryptose sulfite cycloserine agar, Difco TM, BD, Franklin Lakes, NJ) plates, and MRS medium (de Man Rogosa and Sharpe agar, Difco 288130, BD, Franklin Lakes, NJ) to isolate the *C. perfringens* and *Lactobacillus spp.*, respectively. The TSC agar plates and MRS agar plates were then incubated for 48 h at 37°C under anaerobic conditions. Microbial colonies were immediately counted after removal from the incubator and expressed as log10 colony-forming units (CFU) per gram of ileal contents.

**Short chain fatty acid (SCFA) analysis of caecal contents**

To determine SCFA levels, including acetate, propionate and butyrate, 1 g of caecal content was mixed with 4 mL 25% metaphosphoric acid. Samples were centrifuged at 10,000 × g for 20 min and the supernatant was transferred into a 2 mL tube stored at −20°C. The concentration of SCFA was determined by gas chromatography with a flame ionisation detector, fuse silica capillary column and nitrogen was used as the carrier gas. Volatile acid standard mixes (SUPELCO, Bellefonte, PA) were used as standard solutions.

**Morphometric analysis of the small intestine**

At the end of the experiment (day 35), one bird per replicate cage from each treatment group (three birds/treatment in total) was randomly selected and slaughtered by severing the jugular veins. During necropsy, the gastrointestinal tract was removed and a segment of the ileum (from Meckel’s diverticulum to the ileo-caeco-colic junction) was taken from the small intestine. The 3 cm long segments from the centre of each tissue were fixed in 10% formalin for later morphometrical assays. The formalin-fixed gut wall was washed in PBS and embedded in paraffin wax. The 3μm sectioned tissue was stained using haematoxylin and eosin methods. The samples were analysed by light microscopy, and software (Motic image plus 2.0) was used to measure the villus height and crypt depth in fifteen favorably-oriented and representative samples per treatment. The ratio of villus height to crypt depth was also calculated.

**Ammonia analysis of ileal, caecal and faecal contents**

The assay of ammonia analysis was modified from the methods of Weatherburn (1967). One gram of ileal (three replicates with four birds per replicate in each treatment) and faecal (six replicates in each treatment) sample was mixed with 4 mL 25% metaphosphoric acid and centrifuged at 15,000 × g for 10 min. The 1.5 mL of supernatant was transferred into a 2 mL Eppendorf tube and stored at −20°C. 25μL of supernatant was mixed with 1 mL reagent A (5 g of phenol with 250 mg of sodium nitroprusside per litre of solution) and 1 mL reagent B (25 g of sodium hydroxide with 16.8 mL sodium hypochlorite per litre of solution). After waiting 15 min for colour development at 37°C, absorbance was measured at 630 nm. NH₄Cl was used as the standard solution.

**Lactic acid analysis of ileum and caecal contents**

Analysis of lactate was carried out by HPLC (L-4200 UV-VIS Detector, Hitachi, Tokyo, Japan) with Gemini 5 u C6-Phynel 110A 250 × 4.6mm (Phenomenex, Torrance, CL) column. One gram of ileal and caecal sample was mixed with 1 mL de-ionized water and 4 mL cyanide methane, and centrifuged at 4000 × g for 4 min. The supernatant was filtered through a 0.2μm filtration film (Sartorius Minisart, NY 25 single-use syringe filter, Sartorius, Göttingen, Germany) and stored at −20°C. The 10 μL of filtered supernatant was injected into the HPLC to conduct the analysis. The mobile phase was a phosphate buffer (contain 3% methanol) and the rate was set at 1 mL/min. The UV detector wavelength was 220 nm.

**Serum characteristic determination**

Serum biochemical values, including serum glutamic-oxalocetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), cholesterol (CHOL), triglyceride (TG), glucose (GLU), creatinine (CREA), uric acid (UA), globulin (GLO), total-protein (T-P) and albumin (ALB), were measured using an automatic biochemical analyser (Hitachi, 7150 auto-analyser, Hitachi, Tokyo, Japan).

**Statistical analyses**

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

### Results

**Approximately analysis of solid-state fermented wheat bran**

Table 2 presents the approximate analysis of solid-state fermentation wheat bran by $BA$ and $SC$. The results demonstrated that the NDF and ADF levels decreased after solid-state fermentation by $BA$ or $BA$ and $SC$ (NDF: from 38.8% to 18.0 and 18.7%; ADF: from 12.3% to 8.3 and 8.5%). Total dietary fibre of WB, FWBA, and FWBA + SC were 37.4 ± 0.2%, 31.0 ± 1.1% and 33.1 ± 0.8%, respectively. Figure 1 illustrates that $BA$ are able to secrete great amounts of protease, xylanase, and cellulase after 3-day fermentation of WB.

### Growth performance

The effects of 10% WB replacement by FWBA, FWSC and FWBA + SC in diets on the growth performance of 1 to 35-d old broilers are shown in Table 3. From 1 to 21 d, 22 to 35d, and 1 to 35 d of age, there were no significant differences in BW, BWG, FI and FCR among all treatment groups ($p > .05$). From 1 to 35 d, treatments with 10% FWBA and 10% FWBA + SC had better FCR compared to the control treatment (1.41 vs. 1.38 vs. 1.38, $p < .05$).

### Microbial population in ileum

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on microbial population in broiler ileum after 35 d are shown in Table 4. The treatments had no significant effect on Clostridium perfringens ($p > .05$). The treatment with 10% FWBA resulted in significantly higher amounts of lactic acid bacteria in the ileal content compared to the control group ($p < .05$).

### Ammonia in ileum, caecum, and excreta

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on ileal, caecal and faecal ammonia concentrations are shown in Table 5. None of the treatments had a significant effect on ileal, caecal and faecal ammonia levels ($p < .05$).

### Lactic acid in ileum and caecum

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on ileal and caecal lactic acid concentrations are shown in Table 6. There were no significant differences in caecal lactic acid concentration among all treatment groups ($p > .05$). However, the treatment with 10% FWBA and 10% FWSC significantly increased the ileal lactic acid level compared to the control groups ($p < .0001$).

### Short-chain fatty acid ileum and caecum

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on caecal SCFA are shown in Table 7. There were no significant differences in acetic acid, propionic acid or butyric acid among all treatment groups ($p > .05$).

**Table 2.** Approximately analysis and total dietary fibre of solid-state fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

| Item             | WB       | FWBA     | FWSC     | FWBA + SC |
|------------------|----------|----------|----------|-----------|
| DM, %            | 89.3 ± 0.12 | 91.9 ± 0.22 | 93.6 ± 0.00 | 92.2 ± 0.02 |
| CP, %DM          | 19.0 ± 0.11 | 19.0 ± 0.17 | 19.7 ± 0.10 | 18.8 ± 0.00 |
| NDF, %DM         | 38.8 ± 0.01 | 18.0 ± 0.00 | 37.0 ± 0.01 | 18.7 ± 0.00 |
| ADF, %DM         | 12.3 ± 0.34 | 8.33 ± 0.69 | 14.0 ± 0.25 | 8.51 ± 0.39 |
| EE, %DM          | 4.43 ± 0.55 | 3.00 ± 1.27 | 1.95 ± 0.28 | 1.30 ± 0.07 |
| ASH, %DM         | 4.72 ± 0.02 | 4.38 ± 0.37 | 4.50 ± 0.06 | 4.36 ± 0.11 |
| Total dietary fibre, %DM | 37.4 ± 0.20 | 31.0 ± 1.10 | 45.7 ± 0.17 | 33.1 ± 0.80 |

WB: Wheat bran; FWBA: Fermented wheat bran by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat bran by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

Figure 1. Radial diffusion results of wheat bran added 60% water and 10% $BA$ for 3-day fermentation (A) protease (B) xylanase (C) cellulase.
Table 3. Effects of solid-state fermentation by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on growth performance of broilers.

| Experimental period (d) and parameter | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | SEM | p Value |
|---------------------------------------|---------|----------|----------|---------------|-----|---------|
| 1–21 d                                |         |          |          |               |     |         |
| Body weight, g<sup>a</sup>            | 725     | 718      | 728      | 726           | 13.5| .961    |
| Feed consumption, g<sup>b</sup>       | 866     | 826      | 817      | 839           | 13.6| .265    |
| Weight gain, g<sup>b</sup>            | 647     | 642      | 655      | 646           | 14.9| .933    |
| FCR<sup>b</sup>                       | 1.32    | 1.29     | 1.25     | 1.30          | 0.03| .552    |
| 21–35 d                               |         |          |          |               |     |         |
| Body weight, g<sup>a</sup>            | 2043    | 2026     | 1991     | 2053          | 34.9| .627    |
| Feed consumption, g<sup>b</sup>       | 1957    | 1912     | 1886     | 1889          | 49.9| .735    |
| Weight gain, g<sup>b</sup>            | 1318    | 1308     | 1262     | 1327          | 31.8| .517    |
| FCR<sup>b</sup>                       | 1.49    | 1.46     | 1.50     | 1.42          | 0.03| .465    |

Control: 10% wheat bran included; FWBA: Fermented wheat brans by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat brans by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat brans by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

<sup>a</sup>Each value represents the mean of 60 replicates.

<sup>b</sup>Each value represents the mean of 3 replicates (20 birds in each replicate).

FCR: feed conversion ratio.

Table 4. Effects of solid-state fermentation of *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on ileal microbiota concentration of broilers.

| Experimental diets | Item                        | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | SEM | p Value |
|--------------------|-----------------------------|---------|----------|----------|---------------|-----|---------|
|                    | - Log<sub>10</sub> CFU/g    |         |          |          |               |     |         |
|                    | Lactic acid bacteria        | 9.11    | 9.52     | 9.23     | 9.00          | 0.09| .023    |
|                    | Clostridium perfringens     | 8.13    | 8.59     | 8.20     | 8.04          | 0.17| .182    |
|                    | Lactic acid bacteria/Clostridium perfringens | 1.12 | 1.11 | 1.13 | 1.11 | 0.02 | .776 |

Each value represents the mean of 3 replicates with 4 birds in each replicate.

Control: 10% wheat bran included; FWBA: Fermented wheat brans by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat brans by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat brans by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

<sup>a</sup>Means with in the same row with different letters are significantly different (p < .05).

Table 5. Effects of solid-state fermentation of *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on intestinal and faecal ammonia concentration of broilers.

| Experimental diets | Item                | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | SEM | p Value |
|--------------------|---------------------|---------|----------|----------|---------------|-----|---------|
|                    | Ileum<sup>a</sup>   | 18.8    | 30.2     | 29.2     | 26.8          | 3.71| .452    |
|                    | Caecum<sup>a</sup>  | 46.4    | 37.9     | 49.5     | 39.0          | 3.65| .369    |
|                    | Faeces<sup>b</sup>  | 55.4    | 62.1     | 60.0     | 57.1          | 2.74| .513    |

Control: 10% wheat bran included; FWBA: Fermented wheat brans by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat brans by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat brans by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

<sup>a</sup>Each value represents the mean of 3 replicates with 4 birds in each replicate.

<sup>b</sup>Each value represents the mean of 6 replicates.

Table 6. Effects of solid-state fermentation of *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on intestinal lactic acid concentration of broilers.

| Experimental diets | Item    | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | SEM | p Value |
|--------------------|---------|---------|----------|----------|---------------|-----|---------|
|                    | Ileal<sup>a</sup> | 126.6   | 174.4    | 138.2    | 135.1         | 2.90| .0001   |
|                    | Caecal  | 141.6   | 164.6    | 156.5    | 143.4         | 7.55| .176    |

Each value represents the mean of 3 replicates with 4 birds in each replicate.

Control: 10% wheat bran included; FWBA: Fermented wheat brans by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat brans by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat brans by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

<sup>a</sup><sup>b</sup>Means with in the same row with different letters are significantly different (p < .05).
Intestinal morphology

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on the ileal morphology of broilers are shown in Table 8 and Figure 2. There were no significant differences in crypt depth or villi height/crypt depth ratio in the ileum among all treatment groups (p > .05). However, the 10% FWSC group resulted in a higher ileal villi height compared to the control, 10% FWBA and 10% FWBA + SC groups (p < .05).

Serum characteristics

The effects of those diets that had a 10% WB replacement by FWBA, FWSC and FWBA + SC on serum characteristics are shown in Table 9. There were no significant differences in TG, GLU, CREA, UA, GLO, T-P, ALB levels among all treatment groups. However, treatments with 10% FWBA and 10% FWBA + SC had a tendency to reduce serum CHOL compared to the control group (p = .054).

Evaluation of fermented wheat bran meal economic benefit

An evaluation of the economic benefits of adding fermented wheat bran meal to the broiler diet is summarised in Table 10. The income over feed cost (IOFC) of the control and 10% FSBA + SC groups were 45.3 and 47.0 NT$/bird, respectively.

Discussion

In the present study, wheat bran fermentation by a mixture of BA and SC can reduce the NDF from 38.8% to 18.7% and ADF from 12.3% to 8.5%, respectively. In the further determination, total dietary fibre contents in wheat bran were decreased 17.1% or 11.5% by fermentation with BA or fermentation with BA and SC. These results can be attributed to outstanding extracellular cellulase and xylanase produced by Bacillus amyloliquefaciens that we found in the present study (Figure 1). According to the previous study, solid-state fermentation by BA combined with the enzymes production could efficiently degrade non-starch polysaccharides (NSP) to low molecular weight polysaccharides, reducing intestinal viscosity caused by NSP and leading to a higher availability of wheat bran (Castanon et al. 1997; Choct 2006).

Kraler et al. (2014) pointed out that the use of a 15% solid-stated fermented wheat bran by Lactobacillus paracasei and Lactobacillus plantarum included in basal diet could increase coefficients of total tract apparent digestibility of organic matter (2%), crude fibre (9%), ether extract (40%), and ash (14%) in growing pigs. Decreasing NSP and increasing digestibility may the reason that treatments with 10% FWBA and 10% FWBA + SC improved the FCR of
broilers compared to the control group. Nevertheless, Chen et al. (2009) reported fermented total feed by *Bacillus* and *Saccharomyces* had no significant difference on the FCR of broilers. The inconsistent results made clear that whole feed fermentation did not improve nutrient availability while 10% fermentation of wheat bran increased it in our study. Wheat bran contained higher fibre content which induces

![Photomicrography of ileum of 35 d broiler in treatments with control, 10% FWBA, 10% FWSC, and 10% FWBA + SC.](image)

(A) Control (B) 10% FWBA (C) 10% FWSC (D) 10% FWBA + SC. Hematoxylin and eosin stain (40×) (method according to Huang et al. 2012).

Table 9. Effects of solid-state fermentation of *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on serum characteristics of broilers.

| Experimental diets | Item | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC | SEM | p Value |
|--------------------|------|---------|----------|----------|--------------|-----|---------|
|                      | CHOL | 101     | 79\*     | 81\*     | 82\*         | 5.09| .054    |
|                      | TG   | 123     | 100      | 120      | 101          | 11.8| .430    |
|                      | GLU  | 211     | 220      | 215      | 210          | 10.2| .887    |
|                      | CREA | 0.13    | 0.13     | 0.15     | 0.10         | 0.04| .832    |
|                      | UA   | 3.70    | 2.90     | 3.83     | 3.20         | 0.34| .317    |
|                      | GLO  | 2.10    | 2.43     | 2.27     | 2.27         | 0.11| .267    |
|                      | T-P  | 3.07    | 3.17     | 3.17     | 3.13         | 0.05| .528    |
|                      | ALB  | 0.97    | 0.73     | 0.90     | 0.87         | 0.08| .254    |

Each value represents the mean of 3 replicates.
Control: 10% wheat bran included; FWBA: Fermented wheat brans by *Bacillus amyloliquefaciens*; FWSC: Fermented wheat brans by *Saccharomyces cerevisiae*; FWBA + SC: Fermented wheat brans by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*.

\*Means values decreased from control treatment (p < .1).
Wheat bran 7.30, FWBA 8.5, FBSC 8.5, FSBA
maize meal 8.00, soybean meal 14.27, full fat soybean meal 18.3,
monocalcium phosphate 16.95, Calcium carbonate 1.94, L-Lysine-HCl 300,
Item Control 10% FW
BA
10% SC

Table 10. Evaluation of the economic benefit of fermented wheat bran (FWB) supplemented in diet.
| Item             | Control | 10% FWBA | 10% FWSC | 10% FWBA + SC |
|------------------|---------|----------|----------|---------------|
| Feed cost, NT/bird |         |          |          |               |
| 1–35 days        | 45.9    | 44.9     | 44.2     | 44.7          |
| Meat income, NT/bird |       |          |          |               |
| 1–35 days        | 91.2    | 90.5     | 88.9     | 91.7          |
| Income over feed cost, NT/bird | | | | |
| 1–35 days        | 45.3    | 45.6     | 44.7     | 47.0          |

Feed cost: Basing on the costs (NTS/kg) of the ingredients as follows: maize meal 8.00, soybean meal 14.27, full fat soybean meal 18.3, wheat bran 7.30, FWBA 8.5, FBSC 8.5, FSBA + FBSC 8.5, soybean oil 50.0, monocalcium phosphate 16.95, Calcium carbonate 1.94, L-Lysine-HCl 300, D-Methionine 230, salt (NaCl) 2.70, choline chloride, 50% 33.8, vitamin premix 135, and mineral premix 39.0. The fees for processing of basal ration per kg were 16.5 for grain mixture of control group, 16.6 for 10% FWBA group, 16.6 for 10% FBSC group and 16.6 for 10% FSBA + SC group during 1–21 day, respectively. The fees for processing of basal ration per kg were 16.2 for grain mixture of control group, 16.3 for 10% FWBA group, 16.3 for 10% FBSC group and 16.3 for 10% FSBA + SC group during 22–35 day, respectively.

Wheat bran fermentation improves feed availability and results in better growth performance. On the other hand, total feed is made up of high levels of protein and starch which can be easily utilised by microorganisms. Fibre contents in fermented total feed will not be degraded as much as fermented wheat bran. It might be the reason that fermented total feed by BA and SC had no significant influence on FCR. Furthermore, a replacement of 10% of fermented wheat bran in diets could not only reduce the energy required for fermentation, but also reduce feeding expenses while using 10% by-product in broiler diets.

Complex carbohydrates are poorly metabolised by Lactobacillus (Dworkin et al. 2006). However, if complex carbohydrates degraded to low-molecular weight carbohydrates by solid-state fermentation, Lactobacillus could ferment these low-molecular weight carbohydrates to lactic acid (Chen et al. 2013). Therefore, in the present study, we could find out that FWBA group increased Lactobacillus and lactic acid in ileum of broilers. Similarly, Chen et al. (2013) also showed that the Lactobacillus count in the small intestine was higher in fermented feed treatment than in the control treatment in Landes geese. Lactobacillus has been reported to produce a variety of antimicrobial substances such as lactic acid, hydrogen peroxide, reuterin and reutericyclin. These substances can inhibit the growth of many enteric pathogens such as Escherichia coli, Salmonella typhimurium and Staphylococcus epidermidis (Hou et al. 2015).

In the present study, we found that feeding fermented wheat bran did not produce significant differences in caecal microbiota, individual volatile fatty acid and lactic acid content. Kraler et al. (2015) showed that the fermented wheat bran by L. paracasei and L. plantarum had no effects on acetic acid, propionic acid, and butyric acid in the colonic guts of pigs, which is in agreement with our findings. However, Ahmed et al. (2014) found that the supplementation of 2% of Bacillus probiotic powder in broiler diets might enhance nutrient absorption and inhibit pathogenic bacteria that led to a decrease in non-utilised nutrients, which would have been degraded to ammonia by microorganisms in the faeces. In the current study, the counts of Bacillus in the 10% FWBA treatment might not be sufficient to inhibit pathogenic bacteria in intestinal guts and decrease the concentration of ammonia.

Intestinal morphology plays an important role in nutrient absorption. Longer and wider villus measurements indicate a greater intestinal surface area which may lead to higher feed availability. The crypt is known as the villus factory, and deeper crypts suggest faster cell turnover, producing new epithelial cells which migrate along to the top of the villus, compensating for inflammation or sloughing cells damaged by pathogens or bacterial toxins (Yason et al. 1987). We found that the treatment with 10% FWSC could significantly increase ileal villus height and it may be attributed to Saccharomyces cerevisiae. A previous study showed that supplementation of whole yeast or Saccharomyces cerevisiae could improve intestinal morphology (Muthusamy et al. 2011). The cell walls of Saccharomyces cerevisiae, which account for 15 to 25% weight of the cell, are predominantly composed of glucan and mannanproteins, including mannan-oligosaccharide and β-glucan (Lipke & Ovalle 1998). These compounds might avoid pathogenic bacteria binding to the villus and protect mucosa from toxic materials. Therefore, the 10% FWSC group had higher villus height in the ileum of broilers compared to other treatments (Zhang et al. 2005).

In the current study, the results showed that treatments with 10% FWBA, 10% FWSC, 10% FWBA + SC had a tendency to reduce serum cholesterol compared to the control treatment (p = .054). Pereira and Gibson (2002) reported that the cholesterol in animal guts could be taken into the cellular membrane of Lactobacillus. It may follow that wheat bran fermented by Bacillus increased intestinal Lactobacillus to
assimilate cholesterol which might reduce serum cholesterol in broilers. On the other hand, a previous study showed that *Saccharomyces cerevisiae* was able to remove cholesterol in animal guts (Psomas et al. 2003). Paryad and Mahmoudi (2008) also reported that supplementing 1.5% *Saccharomyces cerevisiae* in diets could reduce the serum cholesterol of broilers, which is similar to our results.

**Conclusions**

Solid-state fermented wheat bran with BA degraded NDF, ADF, and total dietary fibre, leading to lower FCR in 10% FWBA and 10% FWBA + SC treatments. The 10% FW group also increased *Lactobacillus* and lactic acid in the ileum, while the 10% FWSC group had a greater ileal villus height. In addition, all of the fermented wheat bran treatments had a tendency to decrease the serum cholesterol of broilers compared to the control group.

**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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