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ORIGINAL ARTICLE

Protein enrichment and digestion improvement of napiergrass and pangolagrass with solid-state fermentation

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Background and Purpose: Napiergrass (*Pennisetum purpureum* Schumacher) and pangolagrass (*Digitaria decumbens* Stent) are two major forage grasses for cow feeding. They possess high yields and high regeneration properties. Inoculation of cellulolytic microbes on herbage could enhance the protein content of herbage and promote digestibility in chickens.
Methods: Cellulolytic microbes were isolated from various sources and cultivated on napiergrass and pangolagrass with solid-state fermentation for protein enrichment and *in vitro* digestion improvement.The fermented napiergrass and pangolagrass were used as the main protein source in chicken diets to assess the feasibility for non-ruminants feed.
Results: After a 42-day fermentation period, napiergrass showed higher protein contents (13.4—13.9%) than those of pangolagrass(11.1—11.7%). The *in vitro* digestibility of pangolagrass increased from 5.29% to 20.4%, whereas that of napiergrass increased from 5.29% to 19.0%. The average feed conversion efficiencies of chickens were close to the traditional fodder using corn as the main ingredient.
Conclusion: Inoculation of appropriate cellulolytic microbes to enrich protein content and improve *in vitro* digestibility of herbage with solid-state fermentation for chicken feed is the prospective technique for agriculture, animal husbandry, and substantial management.

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Introduction

As the food and feed protein shortage has become a global crisis and the protein demands for direct human consumption and animal feeding increase for improving human living standards, the protein production from cellulosic resources for animal feeds are being considered worldwide.[1] In 2011, Taiwan imported 2.34×10^6 tons of soybean and 4.19×10^6 tons of corn from other countries, which cost 1.30×10^9 US dollars and 1.41×10^9 US dollars, respectively.[2] Consequently, it is urgent to develop local protein resources with renewable raw materials for animal feed. Cellulose resources are rich in nature, but protein content and *in vitro* digestion are at low levels.[3,4,5] How to convert the cellulosic materials to animal feed is a potential issue. In recent years, the use of cellulolytic microbes to covert cellulosic materials to non-ruminants feeds was an attractive subject in animal husbandry.[6,7] Napiergrass (*Pennisetum purpureum* Schumacher) and pangolagrass (*Digitaria decumbens* Stent) are two major forage grasses in Taiwan with high yield and high regeneration properties.[2] The yield of napiergrass was between 140.77 and 183.92 ton/ha, the cultivation area ranged from 2184 to 3112 ha, and the annual production was $3.21-5.20 \times 10^5$ tons from the years 2001 to 2010 in Taiwan. Although the yield of pangolagrass was between 68.28 and 80.80 ton/ha, the cultivation area ranged from 2965 to 4817 ha, and the annual production was $2.04-3.86 \times 10^5$ tons.[2] After continuous improvement on and research of the cultivated varieties of napiergrass and pangolagrass over the past 20 years, new applications of these forage grasses, such as antioxidant effects, pharmacologic uses, and alcohol, acetic acid, butanol, and biomass hydrogen productions, were developed.[4,5,8,9] In addition to direct use in animal husbandry, these cellulose-rich herbages can have improved nutrient value by fermentation and supporting the development of substantial agriculture.[1,5,9,10,11,12,13]

Solid-state fermentation is a convenient technique to decompose organic compounds and produce proteins, enzymes, and secondary metabolites by inoculating the microbes on solid substances.[13,14,15,16,17] Advantages of this developed technology are its low cost, easy operation, and variety of uses.[16,18,19] Solid-state fermentation can be performedat industry scale and at the rural level.[20,21] Solid-state fermentation holds tremendous potential for the cellulase fermentation and cellulose bioutilization for low cost and high potency.[22,23] Research has indicated that microorganisms could produce enzymes such as carboxymethyl cellulase (CMCase) and cellobihydrolase to decompose cellulose. The cellulolytic microbes can convert the cellulosic materials to protein and improve the nutrient value of forage. Using these microbes with solid-state fermentation for protein enrichment increases the application values of cellulosic forages.[1,7,16,19,24,25] Improving the efficiency of enzyme secretion such as cellulase, phytase, and xylanase during fermentation could improve the feed digestion ratio of poultry and enhance the application value of feed. Solid-state fermentation has high potential for animal husbandry and food provisions.[25,26,27,28]

The aim of this study is to investigate the potential of cellulase production by microbes and the effect on protein enrichment and *in vitro* digestion of forage grasses with solid-state fermentation. Napiergrass and pangolagrass are the common herbages in Taiwan. We use them as the substrates of solid-state fermentation to enrich protein content and improve *in vitro* digestion by cellulolytic microbes. The average body weight gain, feed intake, and feed conversion ratio of broilers with fermented napiergrass and pangolagrass as the main protein source in chicken diets are also discussed.

Materials and methods

Napiergrass and pangolagrass

Fresh napiergrass was obtained from experimental farms of National Pingtung University of Science and Technology and pangolagrass was supplied by experimental stations of Hsinchu Branch, Livestock Research Institute, Council of Agriculture. Fresh napiergrass contained moisture $65.1 \pm 1.5\%$, crude protein $1.42 \pm 0.07\%$, and ash $5.21 \pm 0.12\%$; fresh pangolagrass contained moisture $65.3 \pm 1.4\%$, crude protein $2.34 \pm 0.09\%$, and ash $4.02 \pm 0.10\%$. After harvest, napiergrass and pangolagrass were dried under sunlight and pulverized to an average length of 2–3 cm. The forage grasses were stored at room temperature for further study.

Tested microbes

More than 200 thermotolerant cellulolytic microbes were isolated from composts, biofertilizers, and soils. Fungal isolate *Entrophospora* sp. NP1 had high avicelase (2.15 ± 0.09 U mL^{-1}), β-glycosidase (4.19 ± 0.09 U mL^{-1}), CMCase (6.24 ± 0.08 U mL^{-1}), xylanase (17.00 ± 0.23 U mL^{-1}), and phytase (22.27 ± 0.42 U mL^{-1}) activity. Bacterial isolate *Bacillus subtilis* H8 also had high avicelase (2.38 ± 0.20 U mL^{-1}), β-glycosidase (4.46 ± 0.18 U mL^{-1}), CMCase (6.56 ± 0.20 U mL^{-1}), xylanase (18.02 ± 0.36 U mL^{-1}), and phytase (21.80 ± 1.70 U mL^{-1}) activity. Therefore, fungal isolate *Entrophospora* sp. NP1 and bacterial isolate *Bacillus subtilis* H8 were selected in this study. Bacteria were cultivated in nutrient agar; fungi were cultivated in potato dextrose agar.

Solid-state fermentation

The protein content of napiergrass and pangolagrass was $1.42 \pm 0.07\%$ and $2.34 \pm 0.09\%$, respectively. The inorganic nitrogen should be supplemented for protein enrichment, and $(NH_4)_2SO_4$ was used to adjust the carbon to nitrogen (C/N) ratio in the range of 10 to 20 for protein enrichment.[13,23,29,30] The basal solid medium comprised napiergrass, 100 g,$(NH_4)_2SO_4$, 4.9 g at pH value 6.8 and moisture content 65%, or contained pangolagrass 100 g, $(NH_4)_2SO_4$, 5.0 g at pH value 6.8 and moisture content 65%. The solid medium was mixed thoroughly with spores or cells (10^7 spores or cells mL^{-1}) that were washed with 5 mL of 0.05% Tween-80 in sterilized water, and incubated statically in a flask (the thickness of medium was about 2 cm) at 30°C for 7–42 days by stirring once a day.[13,31]

In vitro digestibility

Sample powder 1 g was suspended in 14 mL of 0.05 M sodium acetate buffer (pH value 5.5) with xylanase (Sigma-Aldrich, X4001) and cellulase (Sigma-Aldrich, 219466), then added with 54.5 mg of pepsin (Sigma-Aldrich, EC.3.4.23.1) in 2.6 mL of sodium acetate buffer, 1.4 mL of 1 M HCl at pH value 3.0, and 48.1 mg of pancreatin (Sigma-Aldrich, EC 232-468-9) in 1 M $NaHCO_3$ at pH value 6.5. After digestion, the mixture was centrifuged at 12,000 *g* with Sigma 3K20 rotor No. 9137 for 10 minutes. The weight loss during the treatment is the *in vitro* digestibility.[32]

Composition of broiler diets

The broiler diet compositions of chickens at earlier growth period (0—3 weeks) and growing period (4—6 weeks) are listed in Table 1. Total protein content was 22% during the chicken earlier growth period, and it was 20% at chicken growing period. Corn was replaced by fermented napiergrass and pangolagrass as the main protein source in chicken diets.

In vivo digestibility

To investigate the feasibility of using fermented napiergrass and pangolagrass as the main protein source in chicken diets, the powder of fermented napiergrass and pangolagrass substrates was used as the main protein source of broiler diets instead of corn. One-day-old chickens of Arbor-Acres broiler strain were divided into five groups, and each group comprised 10 chickens. The chickens were housed in cages, kept in separate rooms with recommended ambient temperature, and fed freedom takes with diets. During 6 weeks of feeding, the chickens were weighed every week and diet consumption recorded daily.

Average feed conversion ratio

The feed conversion ratio was defined as the ratio of consumed food weight to body weight gained.[33] The average feed conversion ratio was the ratio of average consumed food weight to the average body weight gained of chicken.

Chemical analysis

Moisture content was determined by drying a sample at 105°C for 24 hours to a constant mass. Ash content was measured with air dry sample by heating at 550—600°C for 24 hours.[34] The pH value was measured directly or in five times volume of distilled water with a pH meter (Good digital pH meter, model 2002, Taiwan). Total organic carbon was analyzed by TOC-5000A total organic carbon analyzer (Code HI 8424C, Shimadzu, Japan) and determined as follows. Herbage sample powder 0.3 g, 1 N $K_2Cr_2O_7$ 10 mL, and concentrated H_2SO_4 20 mL were mixed thoroughly and stood statically for 30 minutes. Distilled water 200 mL and 85% H_3PO_4 10 mL were added. After cooling, diphenylamine 1 mL was added as an indicator and the reaction mixture was titrated with 0.5 N of ferrous (II) ammonium sulfate.[34,35,36] Soluble nitrogen was extracted with five times volume of distilled water and shaken for 20 minutes. Soluble and total nitrogen contents were determined by the modified Kjeldahl method,[34,37] and protein content was calculated by 6.25 times the difference between total nitrogen and soluble nitrogen contents of sample.[13]C/N ratio was calculated by the ratio of total organic carbon and total nitrogen contents.[38]

Statistical analyses

Experiments were carried out in triplicate. Statistical analysis was performed according to the SAS User's Guide.[39] One-way analysis of variance was performed, and the difference between specific means was tested for significance by Duncan multiple-range test.[40] The difference between two means was considered statistically significant when $p < 0.05$.

Table 1 Composition of broiler diets

	Control group		Treatment[a]							
			A		B		C		D	
Growth periods (wk)	0—3	4—6	0—3	4—6	0—3	4—6	0—3	4—6	0—3	4—6
Corn (%)	52.4	58.6	—	—	—	—	—	—	—	—
Fermented napiergrass (%)	—	—	62.7	70.1	—	—	62.3	69.6	—	—
Fermented pangolagrass (%)	—	—	—	—	58.4	65.3	—	—	58.4	65.3
Soybean meal (%)	40.5	34.8	30.2	23.3	34.5	28.1	30.6	23.8	34.5	28.1
Soybean oil (%)	4.0	3.5	4.0	3.5	4.0	3.5	4.0	3.5	4.0	3.5
18% Dicalcium phosphate (%)	1.2	1.3	1.2	1.3	1.2	1.3	1.2	1.3	1.2	1.3
35% Limestone (%)	1.1	1.0	1.1	1.0	1.1	1.0	1.1	1.0	1.1	1.0
Salt (%)	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
DL-methionine (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix (%)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
50% Choline-Cl (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[a] Treatment A: Napiergrass fermented with *Entrophospora* sp. NP1. Treatment B: Pangolagrass fermented with *Entrophospora* sp. NP1. Treatment C: Napiergrass fermented with *B. subtilis* H8. Treatment D: Pangolagrass fermented with *B. subtilis* H8.

Results and discussion

Physiochemical properties of napiergrass and pangolagrass during solid-state fermentation

The sunlight dry napiergrass contained total organic carbon 34.7 ± 1.4%, total nitrogen 0.34 ± 0.03%, and C/N ratio 103.6 ± 8.6, whereas the sunlight dry pangolagrass contained total organic carbon 32.8 ± 0.4%, total nitrogen 0.49 ± 0.04%, and C/N ratio 67.5 ± 5.5. Ammonium sulfate was the best inorganic nitrogen source in protein enrichment of sweet potato residue, sugar beet residue, and corncob with solid-state fermentation.[18,22,23,30] Therefore, ammonium sulfate was used as an inorganic nitrogen source to adjust the initial C/N ratio of napiergrass and pangolagrass solid substrates. Properties of napiergrass and pangolagrass solid substrates during fermentation for 42 days are shown in Fig. 1. The pH values of fermentation substrates increased with time during fermentation (Figs. 1A and 1B); this finding might be due to release of NH_4^+ and OH^- with decomposition of nitrogen compounds. Similar phenomena were also found in solid-state fermentation of enzymes, antibiotics, polyunsaturated fatty acids, and biofertilizer production.[8,14,15,31,41,42,43,44] During fermentation, napiergrass and pangolagrass substrates inoculated with *B. subtilis* H8 had the highest pH value, followed by inoculation with *Entrophospora* sp. NP1, and control samples without inoculation showed the lowest pH value. These results indicated that inoculations of *B. subtilis* H8 and *Entrophospora* sp. NP1 on napiergrass and pangolagrass solid substrates would stimulate fermentation and increase the pH value during fermentation.

Moisture content of napiergrass and pangolagrass substrates increased slowly in the early period and had the highest value on the 14th day; moisture content decreased gradually during fermentation (Figs. 1C and 1D). Napiergrass and pangolagrass substrates inoculated with *B. subtilis* H8 and *Entrophospora* sp. NP1 had a higher moisture content than those of control samples without microbial inoculation. The increase in moisture content in the early period was due to the production of metabolic water by microbes, and moisture content decreased gradually after 14 days for the water evaporation during fermentation. These tendencies were the same as solid-state fermentation of enzymes, antibiotics, polyunsaturated fatty acids, and biofertilizers.[8,14,15,31,41,42,43,44]

Ash contents of substrates increased gradually during fermentation, whereas total organic carbon contents decreased gradually (Figs. 1E–1H). Inoculations of *B. subtilis* H8 and *Entrophospora* sp. NP1 enhanced the decomposition of total organic carbons to carbon dioxide and increased ash contents. The same findings were also noted in biofertilizer preparations.[8,17,44]

Total nitrogen content increased gradually during fermentation. After 42 days of fermentation, napiergrass and pangolagrass substrates inoculated with *B. subtilis* H8 and *Entrophospora* sp. NP1 had higher total nitrogen content than those of control samples without microbial inoculation (Figs. 1I and 1 J). C/N ratio decreased from 14.3–14.4 to 10.3–10.4, and from 16.1–18.0 to 12.6–13.6 during fermentation in napiergrass and pangolagrass, respectively (Figs. 1K and 1L). These results were similar to the protein enrichments of sweet potato residue and corncob, biofertilizer productions of livestock, and kitchen and food waste products.[8,17,23,30,44] The soluble nitrogen content of napiergrass and pangolagrass decreased markedly in the early period and then reached a constant value after 21 days of fermentation (Figs. 1M and 1N). The biomass conversion of solid substrate was related to the consumption of soluble nitrogen. Because of the high fermentation activities of inoculation microbes in the initial stage, the soluble nitrogen content was used by microbes, so the of soluble nitrogen content with *B. subtilis* H8 and *Entrophospora* sp. NP1 inoculations was lower than that in the control samples without inoculation.

Protein enrichment of napiergrass and pangolagrass

Protein enrichments of napiergrass and pangolagrass with *B. subtilis* H8 and *Entrophospora* sp. NP1 are shown in Table 2. High fermentation activities in the initial stage resulted in the rapid increase of protein contents. After 42 days of fermentation, napiergrass (13.7 ± 0.82% to 13.9 ± 0.04%) showed higher protein content than that of pangolagrass (11.7 ± 0.26% to 11.8 ± 0.69%). Solid-state fermentation with *B. subtilis* H8 and *Entrophospora* sp. NP1 inoculation could enhance protein content of napiergrass and pangolagrass. However, the results were not significant between two tested inoculates. Similar results were also described by Ugwuanyi et al[25] with protein enrichment of corncob heteroxylan waste slurry by thermophilic aerobic digestion. Protein enrichment might be due to the secretion of enzymes such as cellulase, phytase, and xylanase during the growth of microbes to convert the fiber materials for monosaccharide.[25] High fermentation efficiency and high protein content (13.7% and 13.9%, respectively) were found in napiergrass inoculated with *B. subtilis* H8 and *Entrophospora* sp. NP1 after 42 days of fermentation. In pangolagrass, *B. subtilis* H8 and *Entrophospora* sp. NP1 also enhanced protein content (11.8% and 11.7%, respectively). Biomass conversion by microbes with solid-state fermentation was a potential application technology in animal husbandry. The same phenomena were also shown in cassava and its by-products,[3,12,45] orange waste,[1] and banana peel.[7]

In vitro digestibility of napiergrass and pangolagrass with solid-state fermentation

Many researchers have focused on human digestion and release of foods, drugs, functional foods, and bioactive substances in recent years. The digestion and absorption of these active compounds were estimated by *in vitro* and *in vivo* models to increase the digestion rate and absorption rate, and improve health care or environmental protection.[32,46,47] Table 3 shows the *in vitro* digestibility of napiergrass and pangolagrass during solid-state fermentation. The *in vitro* digestibility increased with solid-state fermentation. Fermented napiergrass had higher *in vitro* digestibility than that of pangolagrass. Fermented napiergrass with *B. subtilis* H8 and *Entrophospora* sp. NP1 for 42 days had *in vitro* digestibility of 24.10 ± 0.38% and

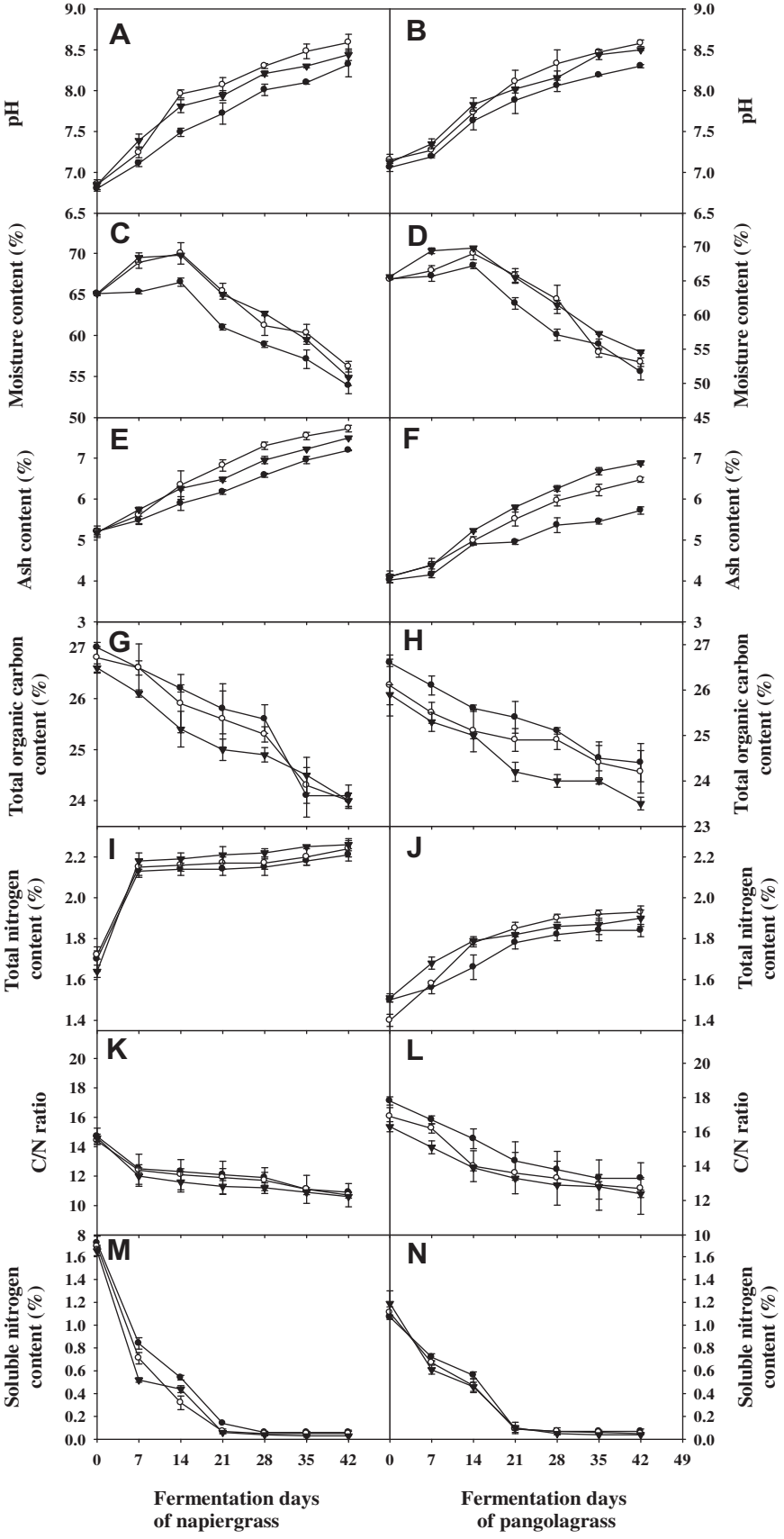

Figure 1. Properties of napiergrass and pangolagrass solid substrates during fermentation for 42 days. Control without microbial inoculation (●), inoculation with *B. subtilis* H8 (○), and inoculation with *Entrophospora* sp. NP1 (▼). Data points are the means and vertical bar indicates the standard deviations ($n \geq 3$).

Table 2 Protein contents of napiergrass and pangolagrass during solid-state fermentation

Incubation periods (day)	Noninoculation control (%)	Microbial inoculation	
		B. subtilis H8 (%)	*Entrophospora* sp. NP1 (%)
(A) Napiergrass			
0	1.42 ± 0.03[a]	1.49 ± 0.06[a]	1.52 ± 0.12[a]
7	8.05 ± 0.54[b]	9.00 ± 0.53[b]	10.40 ± 0.53[c]
14	9.95 ± 0.33[c]	11.50 ± 0.21[c]	11.90 ± 0.43[c]
21	12.50 ± 0.20[d]	13.10 ± 0.80[d]	13.40 ± 0.63[d]
28	13.00 ± 0.20[d]	13.30 ± 0.79[d]	13.70 ± 0.15[d]
35	13.20 ± 0.03[d]	13.40 ± 0.83[d]	13.90 ± 0.07[d]
42	13.40 ± 0.18[d]	13.70 ± 0.82[d]	13.90 ± 0.04[d]
(B) Pangolagrass			
0	2.34 ± 0.06[a]	2.26 ± 0.10[a]	2.21 ± 0.03[a]
7	5.27 ± 0.42[b]	5.70 ± 0.08[b]	6.67 ± 0.45[b]
14	6.86 ± 0.18[c]	8.20 ± 0.36[c]	8.35 ± 0.38[c]
21	10.60 ± 0.69[d]	11.00 ± 0.30[d]	10.80 ± 0.36[d]
28	10.90 ± 0.46[d]	11.40 ± 0.68[d]	11.30 ± 0.02[d]
35	11.10 ± 0.39[d]	11.60 ± 0.68[d]	11.50 ± 0.15[d]
42	11.10 ± 0.29[d]	11.80 ± 0.69[d]	11.70 ± 0.26[d]

Values (means ± standard deviation, $n = 3$) in the same column with different letters are significantly different ($p < 0.05$).

22.00 ± 0.31%, respectively, whereas in fermented pangolagrass it was 20.40 ± 0.28% and 19.00 ± 0.33%, respectively. Control samples without microbial inoculation of napiergrass and pangolagrass had digestibility of only 10.20 ± 0.11% and 9.12 ± 0.29%, respectively. There were significant differences between the microbial inoculation and control samples without inoculation. These results showed that solid-state fermentation of pangolagrass and napiergrass with appropriate inoculation could enhance the *in vitro* digestibility. The increasing *in vitro* digestibility of fermented napiergrass and pangolagrass might be due to high soluble phosphorous content, protein content, and enzyme activities.[13,19,48] Solid-state fermentation of pangolagrass and napiergrass with appropriate microbes could be used in the feed industry for *in vitro* digestibility improvement and certain nutrition source applications.

In vivo digestibility of napiergrass and pangolagrass with solid-state fermentation

Effects of solid-state fermentation on the *in vivo* digestibility of chickens were investigated using the average body weight gain, average feed intake, and average feed

Table 3 *In vitro* digestion of napiergrass and pangolagrass during solid-state fermentation

Incubation periods (day)	Noninoculation control (%)	Microbial inoculation	
		B. subtilis H8 (%)	*Entrophospora* sp. NP1 (%)
(A) Napiergrass			
0	7.10 ± 0.48[a]	6.94 ± 0.21[a]	7.02 ± 0.33[a]
7	7.61 ± 0.18[a]	8.33 ± 0.31[b]	9.01 ± 0.54[b]
14	8.01 ± 0.22[b]	11.20 ± 0.20[c]	10.90 ± 0.19[c]
21	8.13 ± 0.56[b]	13.60 ± 0.41[d]	14.60 ± 0.37[d]
28	9.29 ± 0.27[c]	17.80 ± 0.18[e]	19.60 ± 0.11[f]
35	9.81 ± 0.09[c]	23.20 ± 0.34[f]	21.90 ± 0.23[f]
42	10.20 ± 0.11[c]	24.10 ± 0.38[g]	22.00 ± 0.31[f]
(B) Pangolagrass			
0	5.65 ± 0.08[a]	5.29 ± 0.36[a]	5.82 ± 0.55[a]
7	5.81 ± 0.10[a]	9.12 ± 0.49[b]	7.01 ± 0.20[a]
14	6.33 ± 0.31[a]	13.50 ± 0.42[c]	9.87 ± 0.33[c]
21	7.28 ± 0.48[b]	15.80 ± 0.25[d]	13.60 ± 0.21[d]
28	8.01 ± 0.12[b]	17.80 ± 0.53[e]	16.80 ± 0.42[e]
35	8.88 ± 0.34[b]	19.60 ± 0.22[f]	18.60 ± 0.45[f]
42	9.12 ± 0.29[b]	20.40 ± 0.28[f]	19.00 ± 0.33[f]

Values (means ± standard deviation, $n = 3$) in the same column with different letters are significantly different ($p < 0.05$).

Table 4 The average body weight gain of broilers with fermented napiergrass and pangolagrass feeding

Growth periods (wk)	Control group (g)	Treatments[a]			
		A (g)	B (g)	C (g)	D (g)
0–2	335 ± 22.7^{b}	352 ± 12.8^{b}	274 ± 25.6^{c}	318 ± 49.5^{b,c}	358 ± 48.0^{b}
3–4	844 ± 69.5^{b}	760 ± 63.8^{b}	762 ± 72.3^{b}	826 ± 75.2^{b}	823 ± 76.4^{b}
5–6	804 ± 80.9^{b}	692 ± 93.0^{b}	760 ± 87.0^{b}	832 ± 93.0^{b}	768 ± 94.0^{b}
0–6	1983 ± 80.0^{b}	1804 ± 80.1^{c}	1796 ± 70.3^{c}	1977 ± 84.0^{c}	1949 ± 76.0^{c}
Average daily gain (g/day)	47.2 ± 0.37^{b}	44.0 ± 0.38^{b}	42.8 ± 0.28^{d}	47.1 ± 0.50^{b}	46.4 ± 0.63^{b}

Treatment A: Napiergrass fermented with *Entrophospora* sp. NP1. Treatment B: Pangolagrass fermented with *Entrophospora* sp. NP1. Treatment C: Napiergrass fermented with *B. subtilis* H8. Treatment D: Pangolagrass fermented with *B. subtilis* H8.
Values (means ± standard deviation, $n = 10$) in the same row with different letters are significantly different ($p < 0.05$).

Table 5 The average feed intake of broilers during 42 days of feeding

Growth periods (wk)	Control group (g)	Treatments[a]			
		A (g)	B (g)	C (g)	D (g)
0–2	588 ± 16.6^{b}	651 ± 21.6^{c}	553 ± 27.0^{b}	575 ± 34.4^{b}	629 ± 26.8^{c}
3–4	1588 ± 18.9^{b}	1569 ± 28.8^{b}	1650 ± 28.5^{c}	1601 ± 18.2^{b,c}	1638 ± 51.2^{b,c}
5–6	1591 ± 89.6^{b}	1610 ± 50.3^{b}	1695 ± 48.2^{b}	1655 ± 36.9^{b}	1601 ± 66.2^{b}
0–6	3767 ± 91.2^{b}	3830 ± 78.8^{b}	3898 ± 93.4^{b}	3831 ± 54.1^{b}	3868 ± 35.3^{b}
Average feed intake (g/day)	89.7 ± 0.60^{b}	91.2 ± 0.14^{b}	92.8 ± 1.02^{b,c}	91.2 ± 0.14^{b}	92.1 ± 1.91^{b,c}

[a] Treatment A: Napiergrass fermented with *Entrophospora* sp. NP1. Treatment B: Pangolagrass fermented with *Entrophospora* sp. NP1. Treatment C: Napiergrass fermented with *B. subtilis* H8. Treatment D: Pangolagrass fermented with *B. subtilis* H8.
Values (means ± standard deviation, $n = 10$) in the same row with different letters are significantly different ($p < 0.05$).

conversion ratio of broilers during 42 days of feeding (Tables 4–6). The average daily body weight gains of broilers with fermented napiergrass and pangolagrass for 42 days of feeding were 42.8 ± 0.28 to 47.1 ± 0.50 g. The group receiving fermented napiergrass had slightly higher average daily body weight gains than those receiving fermented pangolagrass, and the differences were significant with *Entrophospora* sp. NP1 inoculation. Napiergrass and pangolagrass inoculation with *B. subtilis* H8 was also associated with slightly higher average daily body weight gains than *Entrophospora* sp. NP1, and the differences were significant. The control group with corn as the protein source instead of fermented napiergrass and pangolagrass had an average daily body weight gain of 47.2 ± 0.37 g. It was slightly higher than that of fermented napiergrass and pangolagrass, and the differences were significant with *Entrophospora* sp. NP1 inoculation and control group with corn as protein source.

The average feed intake of broilers during 42 days of feeding is shown in Table 5. The fermented napiergrass and pangolagrass groups had slightly higher average daily feed intakes (91.2 ± 0.14 g to 92.8 ± 1.02 g) than the control corn group (89.7 ± 0.60 g), but the differences were not significant. The fermented pangolagrass group also had a slightly higher average daily feed intake (92.1 ± 1.91 g to 92.8 ± 1.02 g) than the fermented napiergrass group (91.2 ± 0.14 g to 91.2 ± 0.16 g), and the differences were also not significant.

The average feed conversion ratios of broilers are presented in Table 6. The average feed conversion ratios of fermented napiergrass and pangolagrass were between 1.94 ± 0.07% and 2.17 ± 0.07%, respectively, and that of the control group with corn as the main ingredient was 1.90 ± 0.08%. Fermented napiergrass and pangolagrass with *B. subtilis* H8 had a lower average feed conversion ratio than that of *Entrophospora* sp. NP1. The fermented

Table 6 The average feed conversion ratio of broilers during 42 days of feeding

Growth periods (wk)	Control group (%)	Treatments[a]			
		A (%)	B (%)	C (%)	D (%)
0–2	1.76 ± 0.07^{b}	1.85 ± 0.05^{b}	2.02 ± 0.09^{c}	1.81 ± 0.05^{b}	1.76 ± 0.12^{b}
3–4	1.88 ± 0.02^{b}	2.06 ± 0.12^{c}	2.17 ± 0.11^{c}	1.94 ± 0.04^{b}	1.99 ± 0.10^{b,c}
5–6	1.98 ± 0.10^{b}	2.33 ± 0.16^{c}	2.23 ± 0.13^{c}	1.99 ± 0.08^{b}	2.08 ± 0.14^{b,c}
0–6	1.90 ± 0.08^{b}	2.07 ± 0.10^{b,c}	2.17 ± 0.07^{c}	1.94 ± 0.07^{b}	1.98 ± 0.06^{b,c}

[a] Treatment A: Napiergrass fermented with *Entrophospora* sp. NP1. Treatment B: Pangolagrass fermented with *Entrophospora* sp. NP1. Treatment C: Napiergrass fermented with *B. subtilis* H8. Treatment D: Pangolagrass fermented with *B. subtilis* H8
Values (means ± standard deviation, $n = 10$) in the same row with different letters are significantly different ($p < 0.05$).

napiergrass and pangolagrass average feed conversion ratios of chickens were higher than the traditional fodder using corn as the main ingredient.

The *in vivo* results were coincident with the *in vitro* investigations (Table 3) that the digestibility of napiergrass and pangolagrass increased with inoculation microbes during solid-state fermentation. Fermented napiergrass and pangolagrass as the main protein source or as partial replacement of corn protein of feed would be a low-cost and environmental protection item. These results demonstrated that using *B. subtilis* H8 and *Entrophospora* sp. NP1 as the inoculating microbes with solid-state fermentation of napiergrass and pangolagrass could enrich protein content and improve *in vitro* digestibility. Inoculation of appropriated microbes in solid substrates could increase the conversion rates, protein contents, and *in vitro* digestibility. The fermented forage grass with solid-state fermentation as the main protein source of fodder is a potential process on decreasing the feed cost and protecting our environment.

These results suggested that napiergrass and pangolagrass, the most common forage grasses in Taiwan, could be potential solid substrates for protein resources with solid-state fermentation. Solid-state fermentation of herbage with appropriate microbes increases protein content and improves *in vitro* digestibility. The average body weight gain and the average feed conversion ratio of broilers with fermented napiergrass and pangolagrass were similar to that of corn as the main protein feed. Inoculation of appropriate microbes with solid-state fermentation to convert the herbage for chicken feed is the prospective technique for agriculture, animal husbandry, and substantial management.

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