



## Research article

# Effect of fiber source diet supplement on growth performance, carcass quality, oxidative stress and intestinal morphology in Thai native chicken (Pradu Hang dum)

Patcharee Kanjak<sup>1</sup>, Wanaporn Tapingkae<sup>1</sup>, Chompunut Lumsangkul<sup>1</sup>, Tossapol Moonmanee<sup>1</sup>, Wipasiri Chaiphun<sup>1</sup>, Suwit Chotinun<sup>2</sup>, Mongkol Yachai<sup>3</sup> and Montri Punyatong<sup>1,\*</sup>

<sup>1</sup>Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup>Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

<sup>3</sup>Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand.

## Abstract

Dietary fiber was used for reduce feed cost in local animal production. However, other advantages effects were interesting to study. Therefore, the objective of this research was to investigated effect of fiber source diet on performance, carcass quality, oxidative stress and intestinal morphology in Thai native chicken. A total of 300 one-day-old (mixed sex) crossbred native chicks (Pradu Hang dum) were randomly assigned to 5 treatments in completely randomized design (CRD). Each treatment had 10 replicates with the crossbred native chickens 6 bird/replicates (1 to 12 weeks of age) were given different levels of banana stem and Napier grass. Treatment diets received as follows a basal diet; 100%, no supplement or control group (CON), basal diet; 95%, 90% supplemented with the banana stem at 50 g/kg, 100 g/kg (BS 5%, BS 10%) of diet, respectively, and basal diet; 95%, 90% supplemented with the Napier grass at 50 g/kg, 100 g/kg (NG 5%, NG 10%) of diet, respectively. The results of the study showed that the use of fiber source: banana stem at 50 g/kg and Napier grass 100 g/kg of the diet improved growth performance. The result showed benefits to increase body weight, improve feed conversion ratio (FCR) and feed cost per gain (FCG), improve to gut health by increase villus height (VH), Villus width (VW) in duodenal and jejunal small intestine morphology, reduced malondialdehyde (MDA) levels in serum ( $p < 0.001$ ). In addition, significantly higher thigh meat percentage ( $p < 0.05$ ) without effecting visceral weight and overall carcass percentage.

**Keywords:** Banana stem; Napier grass; Performance; Oxidative stress; Intestinal morphology; Chicken

**Corresponding author:** Montri Punyatong, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50290, Thailand, E-mail: montri.pun@cmu.ac.th. Tel: +66-62459-7474

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## INTRODUCTION

The world livestock industry has grown quickly in recent decades. Poultry feed production group ranks first based on species in global feed production. According to the Alltech Global Feed Survey (2021) report, the production of broiler chicken feed, which was 332.5 million tons in 2019 and reached 334.5 million tons in 2020. This in turn had raised the need for feedstuffs. However, conventional feedstuffs' yields, particularly those of corn, fish meal, and soybean meal, are insufficient, and their prices have been rising steadily as well (Khempaka et al., 2021). Therefore, research on unconventional feedstuffs may be able to offer solutions to these issues. Simultaneously, livestock sector in Thailand has the requirement of the initiative is to change native chicken production and improve the 56 chances of community careers (Chaikuad et al., 2023) with increased performance of chicken to solve low potential and expansion of native chickens from only in backyard farms up to use in commercial farms for increased chicken production and must have a low cost.

Crossbred native chickens gain favor from consumers due to their tight texture, better flavor and to be healthier than broiler meat (low fat, cholesterol, and triglyceride content) (Phianmongkhol et al., 2012). Most of them were raised by small farmers, who often used other types of chicken feed, it may cause slow growth with poor performance (Kridtayopas et al., 2019). Although, native chickens can adapt well to the environment (Rajpura et al., 2010). However, the major problem in raising chickens in Thailand is the temperature of the environment that is higher than the temperature that the chickens can live comfortably. As a result, the chickens are heat stress. Especially in the summer when the weather is very hot (Maximum temperature from 40 °C above) in many areas of the north, including some areas of the northeastern and central regions (Meteorological Department, 2021) which caused the chicken industry to suffer from heat stress (Bonnet et al., 1997). According to the study of Leotaragul et al. (1996) was found that native chickens raised in the summer will be productivity lower than winter and rainy season (age 8 weeks above) for example, decreased performance and pectoral muscles (Temim et al., 2000; Deeb et al., 2002; Mashaly et al., 2004) and increased mortality (Mahmoud et al., 2004). In addition, the current problems in terms of stress such as environmental stress, management that lacks consideration for animal welfare, the stress of inadequate feed and social hierarchy (Wong et al., 2017; Gismervik et al., 2020; Loengbudnark et al., 2023) may cause the immune system to deteriorate and lead to illness of animals. To resolve this problem, several approaches have been considered including in animal nutrition (Kiczorowska et al., 2017; He et al., 2021).

Although, Fiber or substances high in fiber have historically had a negative impact on the performance of chicken (Janssen and CarrÉ, 1985) but these initial findings refuted by more recent studies. For example, the addition of low dosages of insoluble dietary fiber (DF) either had no effect or improved weight gain, feed intake, feed conversion ratio (FCR), digestibility, and gastrointestinal tract development (Shirzadegan and Taheri, 2017). According to Jiménez-Moreno et al. (2013) reported that a small amount of fiber (about 2.5%) increased the FCR of 1- to 18-day-old chicks without influencing the digestibility of dry matter (DM). Most of the fiber sources previously assessed were by-products from the agriculture, by their composition varied depending on the specie (Montagne et al.,

2003). Dietary fiber is an inherent compound found in common vegetables and grasses. According to the Department of Livestock Development (2008), banana tree products are considered raw materials to developed raise animals very well. Due to the large amount of production each year. There are up to 80% of the leftovers such as banana leaves, banana stem, rhizomes of banana plants. Ripe banana peels, banana blossoms, and banana peels, etc. Banana stem contains high amount of water content (93.4%), banana plants (*Musa cavendishi*) stems had crude protein (CP) of 7.2, crude fiber (CF) of 31.5%, total ash of 21.4%, and neutral detergent fiber (NDF) of 67.2% (DM basis). Acid detergent fiber (ADF), 45.3 percent; hemicellulose, 21.9 percent; cellulose, 35.9 percent; and lignin, 9.4 percent; made up the fibers. Tannic acid equivalent as a percentage of tannin was 0.74 (Viswanathan et al., 1989).

In addition, previous studies suggest that planting the fresh cut grass should also be planted as a supplement to the chickens. The ideal plant for fresh, cutting is Napier grass. Due to high yield and high nutritional value (Nantasaksiri et al., 2021). Napier or elephant grass is a species native in Thailand and Napier Pakchong 1 grass (*Pennisetum purpureum* x *Pennisetum americanum*) is a hybrid variety of that plant. Ahamed et al. (2021) reported that crude protein content of Napier Pakchong 1 grass on cutting periods 40, 50, 60 days were 11.23, 9.49 and 8.01%, respectively. And from reported of Liman et al. (2022) at 40 days of harvesting age, the neutral detergent fiber (NDF) content was 46% and increased at 50, 60 and 70 days of harvesting, 49, 55 and 53%, respectively.

However, the above is an interesting introduction to the raw materials of banana plants and Napier grass. More studies are still needed to determine whether it can be used effectively in animal husbandry. Therefore, the objective of this study was to evaluate the effect of the fiber source supplement on growth performance, carcass quality, oxidative stress and intestinal morphology in the Thai native chicken (Pradu Hang dum).

## MATERIALS AND METHODS

### Animal and experimental design

All experiments were conducted according to the guidelines established for the care and use of laboratory animals, and have been approved by Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Thailand. The ethical approval number AG02001/2566.

A total of 300 one-day-old (mixed sex) crossbred native chicks (Pradu Hang dum) were used in this experiment. On arrival, the chicks were weighed; sexed, wing banded and assigned to treatment groups so that the initial weight was similar among different treatment groups. Male and female chicks were mixed at a ratio of 1:1. Ten replicate pens of 6 chicks were randomly allotted to each of 5 treatments based on a completely randomized design (CRD). The experimental design was randomized throughout (Completely Randomized Design; CRD) there are 5 groups (Treatments; T). Each treatment had 10 replicates with the crossbred native chickens 6 birds/replicates (1 to 12 weeks of age) were given different levels of banana stem and Napier grass. Treatment diets received as follows a basal diet; 100%, no supplement or control group (CON), basal diet; 95%, 90% supplemented with the banana stem at 50 g/kg, 100 g/kg (BS 5%, BS 10%) of diet, respectively, and basal diet; 95%, 90%

supplemented with the Napier grass at 50 g/kg, 100 g/kg (NG 5%, NG 10%) of diet, respectively. All chicks were supplied with feeds and water *ad libitum*.

The diets were formulated to meet the nutrient requirements for day-old broiler chicks according to the nutrient requirements for poultry in line with the agricultural (Dale, 1994) (Table 1). The supplementation of raw materials of banana stem (BS) and Napier grass (NG) is chopped fresher and not more than 5 cm in size. Basal diet, BS and NG was incubated for 3 hours at 60°C and analyzed for proximate components. DM, crude protein (CP), crude fiber (CF), ether extracts (EE), and ash were determined according to the standard procedures (Helrich, 1990).

### Growth performance and carcass quality

**Table 1** Ingredients and chemical composition of diets.

Ingredients	Percent inclusion <sup>1</sup>	
	0-3 weeks	4-12 weeks
Yellow corn	54.97	69.04
Fine rice bran	11.99	10.00
Soybean meal, 44% CP	19.99	12.16
Full fat soybean (FFSB)	5.00	2.94
Meat meal, 50% CP	5.00	3.00
Monocalcium phosphate, 22% P	1.70	1.13
Limestone	0.80	1.13
DL-Methionine	0.10	0.10
Salt (NaCl)	0.20	0.25
Premix <sup>2</sup>	0.25	0.25
Chemical composition analysis (% DM basis)		
Dry matter; DM (%)	90.04	90.61
Ash (%)	9.65	8.26
Crude protein; CP (%)	21.06	14.83
Crude fiber; CF (%)	3.90	3.83
Ether extract; EE (%)	4.24	4.40
Nitrogen free extract; NFE (%)	51.19	59.29
Gross energy; GE (kcal/kg)	3,963.12	3,845.61

<sup>1</sup> As-fed basis.

<sup>2</sup> Premix: Each 1 kg contained 15,000 IU vitamin A, 3,000 IU vitamin D<sub>3</sub>, 25 IU vitamin E, 5 mg vitamin K<sub>3</sub>, 2 mg vitamin B<sub>1</sub>, 7 mg vitamin B<sub>2</sub>, 4 mg vitamin B<sub>6</sub>, 25 mg vitamin B<sub>12</sub>, 11.4 mg pantothenic acid, 35 mg nicotinic acid, 1 mg folic acid, 15 µg biotin, 250 mg choline chloride, 1.6 mg Cu, 60 mg Mn, 45 mg Zn, 80 mg Fe, 0.4 mg I, and 0.15 mg Se.

During the experiment, feed intake and body weight were measured weekly, body weight gain (BWG) and feed intake (FI) of chicks in each pen was recorded at different weeks of the experiment. In addition, performance indices were calculated as following: BWG = final BW-initial BW, Feed conversion ratio (FCR) = FI/BWG, ADG = (Final BW – Initial BW) / Days on test, FCR was also calculated, then use the production efficiency data to calculate the Feed cost per gain (FCG) = FI × cost of feed (THB/kg). Mortality and culling rates as well as abnormal symptoms were recorded immediately at the notice (El-Katcha et al., 2021).

At 12 weeks of age, after fasting for 24 hours before slaughter, 10 randomly birds of each treatment were individually weighted (total of 50 birds), slaughtered by hand using conventional neck cut for bleeding. Scalding was operated at 60°C for 120 seconds and manually eviscerated. Hot carcass was processed into Thai style retailed cut and weighted including skeletal frame, leg (drumstick with thigh), wing, breast with fillet, liver, gizzard, spleen and heart. Hot carcass percentage was calculated as percentage of live body weight. The weight percentages of the cuts were calculated as percentage of hot carcass weight (Pripwai et al., 2014)

### **Oxidative stress determination and sample collection**

Five birds from each group were selected for blood collection at weeks 4, 8 and 12. The serum was separated to measure malondialdehyde level (MDA) (Santos et al., 1980) and glutathione level (GSH) (Boyne & Ellman, 1972) MDA was determined by adding 0.45 ml of normal saline solution (NSS), 0.2 ml of thiobarbituric acid reagent (TBA) and 1 ml of trichloroacetic acid reagent (TCA) to 0.1 ml of serum sample in a test tube. The solution was boiled in water bath at 100°C for 30 minutes. Then 2 ml of distilled water was added and centrifuged at 3,000g for 10 minutes. The supernatant was collected and absorbance was taken at 532 nm, compared with MDA standard for calculated MDA level in sample. GSH was measured by adding 1.6 ml of distilled water to 0.4 ml of serum sample. Then 3 ml of precipitation solution was added for protein precipitation. Then, centrifuged at 2,000g for 10 minutes. Supernatant was collected and 4 ml of phosphate buffer and 0.5 ml of dithiobisnitrobenzoic acid (DTNB) was added to determine the absorbance at 412 nm compared with GSH standard for calculated the GSH level in sample.

### **Intestinal morphology**

After blood collection, birds were dissected and about 2 cm segment from the duodenum, jejunum, ileum were trimmed and used for morphological indices. Samples of tissue were washed with saline then fixed in formalin 10% for 48 hours (El-Katcha et al., 2021) After fixation, the tissue specimens underwent the tissue processing that consisted of 3-step process such as dehydration, clearing, and impregnation with paraffin wax. Paraffin embedded tissues were cut into 5 µm thick sections (3 cross sections/sample) using a rotary microtome and placed on glass slides. Slides were prepared and routinely stained with hematoxylin and eosin (H&E) (Suvarna et al., 2018). The H&E-stained histological slides were observed and visualized using a light microscope optical microscope (ZEISS Axio Lab.A1, ZEISS Industrial Metrology, Germany) equipped with a digital video camera (IMTcamUSB3.0\_ISP6.3, IMT i-Solution Inc.), at 5x and 10x objective lens. After that, IMT i-Solution lite imaging software was used for a quantified morphometric analysis (villus height, villus width, crypt depth, muscularis mucosae thickness, villus height per crypt depth ratio, and villus surface area) (Abdelqader et al., 2013).

### **Statistical analyses**

Data were statistically analyzed using SPSS software (SPSS Inc., Chicago, IL, USA). A completely randomized design by analysis of variance

(ANOVA). Differences between mean values of the treatments were determined using Duncan’s multiple-range test. Differences were considered significant when  $P < 0.05$ . All mean values for each parameter within the different treatments were reported as  $\pm$  standard error of the means (SEM).

## RESULTS

### Nutritional values

The nutritional values of banana stem (BS) and Napier grass (NG) are presented in Table 2. The chemical compositions of BS included dry matter (5.14%), crude protein (8.29%), ash (10.76%), crude fiber (20.63%), total fat (2.41%) and NG included dry matter (15.7%), crude protein (11.18%), ash (9.61%), crude fiber (22.64%), and total fat (3.09%), respectively.

**Table 2** The chemical composition of banana stem and Napier grass.

Chemical composition	Banana stem	Napier grass
Dry matter; DM (%)	5.14	15.72
Crude protein; CP (%)	8.29	11.18
Ash (%)	10.76	9.61
Crude fiber; CF (%)	20.63	22.64
Ether extract; EE (%)	2.41	3.09

### Growth performance

The results of the effect of the fiber source diet supplement on growth performance during the experimental period (1 to 12 weeks of age) are presented in Table 3. The present study showed that no significant differences ( $p > 0.05$ ) in initial weight, feed intake among dietary groups. While, the final weight, BWG, ADG were increased among the groups the birds that were fed the fiber source diet supplement when compared with that of the control group ( $p < 0.001$ ). FCR was significantly improved in BS 10%, NG 5%, and NG 10% fed birds compared with the control and BS 5% ( $p < 0.05$ ). However, when considering the cost, FCG was significantly decreased among the birds that were fed the BS 10% and NG 5% diets supplement compared with the NG 10%, BS 5% and control ( $p < 0.05$ ).

**Table 3** Effect of fiber source diet supplement on growth performance of Thai native chicken (Pradu Hang dum).

Parameters	CON <sup>2</sup>	BS 5%	BS 10%	NG 5%	NG 10%	SEM <sup>3</sup>	<i>p</i> -value
Initial weight (g)	150.27±4.43	149.11±8.78	155.69±9.98	151.41±6.84	156.33±6.57	1.11	0.135
Final weight (g)	1,013.50±11.18 <sup>b</sup>	1,139.33±13.65 <sup>a</sup>	1,166.67±15.90 <sup>a</sup>	1,158.33±16.65 <sup>a</sup>	1,125.00±11.79 <sup>a</sup>	12.37	< 0.001
BWG <sup>1</sup> (g)	863.23±11.98 <sup>b</sup>	990.22±19.00 <sup>a</sup>	1,010.98±12.38 <sup>a</sup>	1,006.92±16.33 <sup>a</sup>	968.67±10.94 <sup>a</sup>	12.33	< 0.001
ADG (g/b/d)	12.33±0.17 <sup>b</sup>	14.15±1.70 <sup>a</sup>	14.44±1.46 <sup>a</sup>	14.38±0.66 <sup>a</sup>	13.84±0.16 <sup>a</sup>	0.18	< 0.001
FI (g/b)	4,757.44±226.40	5,037.34±308.09	4,942.86±343.72	4,950.00±108.01	4,930.02±115.92	35.12	0.146
FCR	5.00±0.24 <sup>a</sup>	4.74±0.60 <sup>ab</sup>	4.50±0.41 <sup>b</sup>	4.53±0.18 <sup>b</sup>	4.66±0.13 <sup>b</sup>	0.05	0.022
FCG* (THB/kg)	71.50±3.39 <sup>a</sup>	67.84±4.58 <sup>ab</sup>	64.81±5.85 <sup>b</sup>	66.64±2.60 <sup>b</sup>	66.64±1.89 <sup>ab</sup>	0.79	0.036

<sup>a,b</sup> Mean values with different letters in the same row indicate significant differences ( $p$ -value < 0.05). <sup>1</sup>BWG: body weight gain; ADG: average daily weight gain; FI: feed intake; FCR: feed conversion ratio, FCG: feed cost per gain, THB: currency abbreviation for the Thai baht; <sup>2</sup>CON: control diet; BS 5%: basal diet supplemented with the banana stem at 50 g/kg of diet; BS 10%: banana stem supplementation at 100 g/kg of diet; NG 5%: basal diet supplemented with the Napier grass at 50 g/kg of diet; NG 10%: basal diet supplemented with the Napier grass at 100 g/kg of diet; <sup>3</sup> SEM: standard error of the mean; \* The cost of feed was 14.3 THB/kg.

## Carcass quality

The effects of the fiber source diet supplement on carcass quality and relative organ weight are presented in Table 4. There were no significant differences ( $p > 0.05$ ) in live body weight, featherweight and the carcass percentage among all groups. In cut-up parts, the weight of the thigh was significantly ( $p < 0.001$ ) higher in NG 5% diets supplemented compared with the other groups. While, the spleen weight was increased among the groups the birds that were fed the fiber source diet supplement when compared with the control group ( $p < 0.05$ ). In addition, the proventriculus weight was higher in the CON, BS 10% and NG 10% diet group when compared with the BS 5% and NG 5% ( $p < 0.05$ ).

**Table 4** Effect of fiber source diet supplement on carcass quality and relative organ weight of Thai native chicken (Pradu Hang dum).

Parameters	CON <sup>1</sup>	BS 5%	BS 10%	NG 5%	NG 10%	SEM <sup>2</sup>	p-value
Live body weight (kg)	1.11±0.20	1.31±0.26	1.23±0.18	1.27±0.16	1.18±0.19	0.03	0.281
Defeather weight (kg)	0.97±0.16	1.12±0.23	1.04±0.19	1.18±0.10	1.09±0.16	0.03	0.170
Carcass percentage (%)	73.33±4.64	74.46±2.23	72.65±7.98	75.68±6.67	73.84±6.10	0.81	0.816
Inner organ (% live weight)	12.22±1.63	11.83±1.32	12.15±2.11	11.33±1.34	11.85±3.04	0.27	0.871
Breast	8.83±1.08	8.37±0.84	10.06±2.04	9.42±2.28	10.87±2.94	0.30	0.064
Thigh	12.00±2.69 <sup>b,c</sup>	11.20±1.47 <sup>c</sup>	14.36±3.53 <sup>b</sup>	16.34±4.58 <sup>a</sup>	14.21±3.80 <sup>b</sup>	0.69	< 0.001
Liver	2.37±0.53	2.05±0.34	2.44±0.49	2.21±0.54	2.48±0.70	0.02	0.490
Spleen	0.30±0.27	0.33±0.15	0.40±0.14	0.39±0.14	0.66±0.20	0.02	0.618
Kidney	0.62±0.13	0.37±0.10	0.50±0.12	0.41±0.16	0.60±0.25	0.19	0.158
Heart	0.54±0.14	0.46±0.09	0.51±0.20	0.50±0.14	0.53±0.14	0.02	0.518
Proventriculus	0.74±0.22 <sup>a</sup>	0.63±0.17 <sup>b</sup>	0.71±0.14 <sup>a,b</sup>	0.63±0.12 <sup>b</sup>	0.70±0.23 <sup>a</sup>	0.03	0.004
Gizzard	3.01±0.92	2.96±0.59	4.03±1.05	3.17±1.09	3.99±1.51	0.16	0.065
Intestine	5.56±0.92	4.79±0.86	5.12±1.31	4.07±1.36	5.00±1.83	0.42	0.071
Wing	10.21±2.36	9.48±1.33	10.93±2.44	10.24±2.30	12.00±3.45	0.35	0.246
Leg	5.04±1.37	4.36±0.97	5.36±1.42	4.72±1.15	5.46±1.84	0.20	0.447

<sup>a,b</sup> Mean values with different letters in the same row indicate significant differences ( $p$ -value < 0.05). <sup>1</sup> CON: control diet; BS 5%: basal diet supplemented with the banana stem at 50 g/kg of diet; BS 10%: banana stem supplementation at 100 g/kg of diet; NG 5%: basal diet supplemented with the Napier grass at 50 g/kg of diet; NG 10%: basal diet supplemented with the Napier grass at 100 g/kg of diet; <sup>2</sup> SEM: standard error of the mean.

## Oxidative stress

Oxidative status of the Thai native chicken (Pradu Hang dum) was shown in Table 5. MDA level in serum of birds fed the fiber source diet supplement all levels were lower than control group ( $p < 0.001$ ). GSH level in serum of NG 10%, NG 5%, BS 5% was higher than control group ( $p < 0.001$ ). However, no difference between BS 10% and control groups.

**Table 5** Effects of fiber source diet supplement on oxidative status in serum of Thai native chicken (Pradu Hang dum).

Parameters	CON <sup>1</sup>	BS 5%	BS 10%	NG 5%	NG 10%	SEM <sup>2</sup>	p-value
<b>Malondialdehyde; MDA (µg/ml)</b>							
Week 4	12.64±1.41 <sup>a,b</sup>	9.95±1.90 <sup>d</sup>	13.03±1.83 <sup>b</sup>	11.28±1.04 <sup>c</sup>	13.02±0.38 <sup>b</sup>	0.71	< 0.001
Week 8	13.25±3.06 <sup>a</sup>	10.86±1.90 <sup>d</sup>	11.51±0.87 <sup>c</sup>	12.21±0.70 <sup>b</sup>	12.62±0.70 <sup>b</sup>	0.58	< 0.001
Week 12	24.52±7.80 <sup>a</sup>	13.78±2.83 <sup>c,d</sup>	16.21±1.94 <sup>b,c</sup>	17.55±0.96 <sup>b</sup>	15.16±3.56 <sup>b</sup>	0.74	< 0.001
<b>Glutathione reduced form; GSH (µg/ml)</b>							
Week 4	295.13±60.91 <sup>b,c</sup>	306.93±64.09 <sup>c</sup>	303.33±68.48 <sup>b</sup>	375.27±56.72 <sup>a</sup>	438.53±63.03 <sup>a</sup>	25.45	< 0.001
Week 8	532.13±97.63 <sup>c</sup>	580.00±30.18 <sup>b</sup>	529.52±92.40 <sup>c</sup>	705.33±70.39 <sup>a</sup>	461.67±45.55 <sup>c</sup>	41.89	< 0.001
Week 12	724.09±46.43 <sup>b,c</sup>	1,026.07±90.12 <sup>a</sup>	912.07±96.47 <sup>b</sup>	1,157.93±65.22 <sup>a</sup>	1,231.27±75.48 <sup>a</sup>	74.41	< 0.001

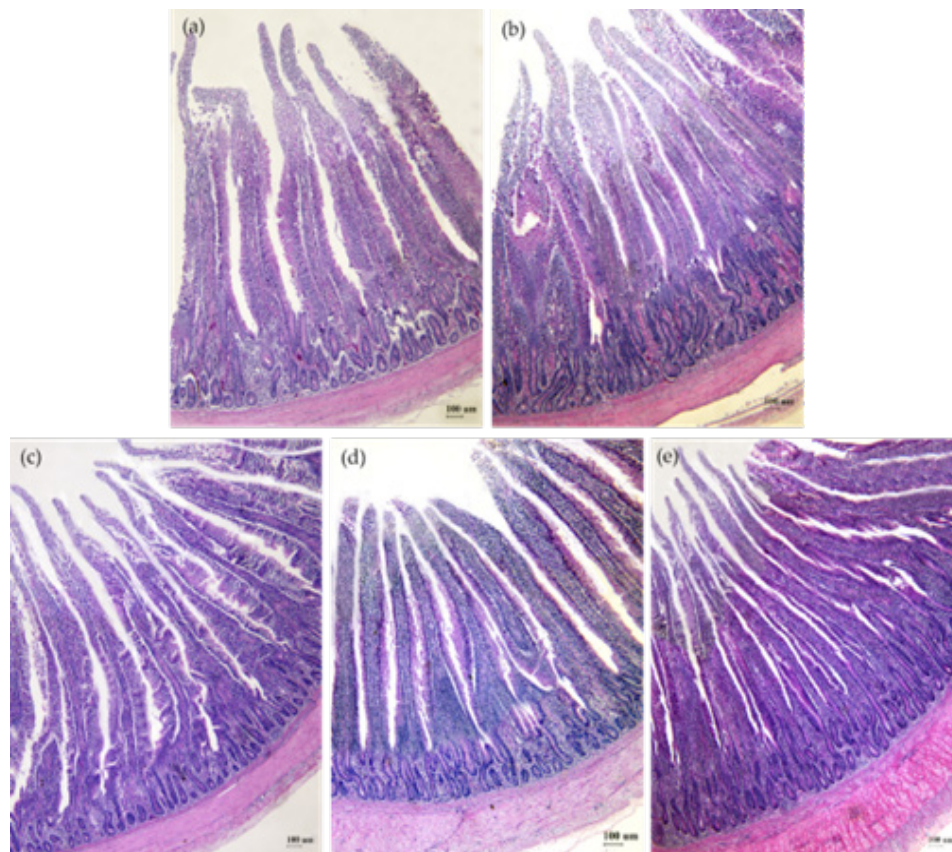
<sup>a-d</sup> Mean values with different letters in the same row indicate significant differences (*p*-value < 0.05). <sup>1</sup> CON: control diet; BS 5%: basal diet supplemented with the banana stem at 50 g/kg of diet; BS 10%: banana stem supplementation at 100 g/kg of diet; NG 5%: basal diet supplemented with the Napier grass at 50 g/kg of diet; NG 10%: basal diet supplemented with the Napier grass at 100 g/kg of diet; <sup>2</sup> SEM: standard error of the mean.

**Table 6** Effect of fiber source diet supplement on small intestinal morphology in Thai native chicken (Pradu Hang dum) at 10 weeks of age.

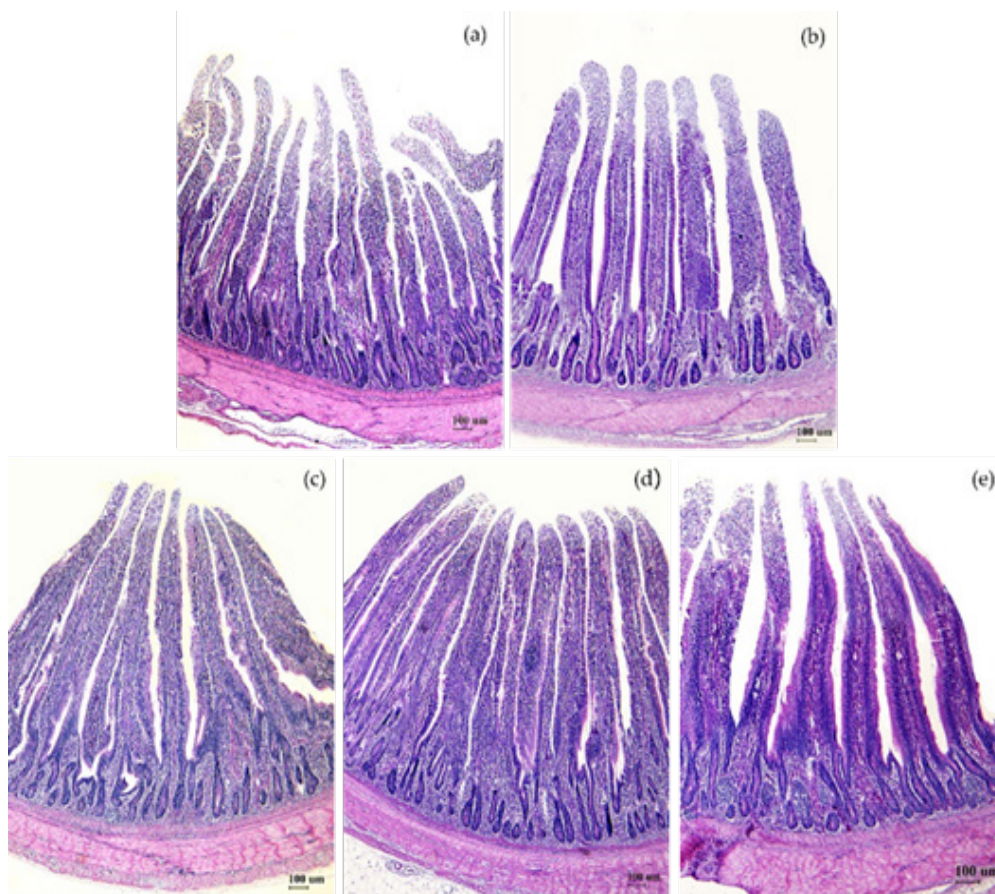
Parameters	CON <sup>1</sup>	BS 5%	BS 10%	NG 5%	NG 10%	SEM <sup>3</sup>	p-value
<b>Duodenum morphology</b>							
Villus height; VH (µm)	1,974.99 ±149.48 <sup>b</sup>	2,032.17 ±267.97 <sup>b</sup>	1,990.88 ±209.48 <sup>b</sup>	2,158.61 ±173.82 <sup>a,b</sup>	2,297.12 ±243.67 <sup>a</sup>	32.64	0.004
Villus width; VW (µm)	297.80±36.11	286.32±45.19	277.01±43.26	279.32±46.23	298.16±27.33	5.60	0.655
Crypt depth; CD (µm)	251.43±25.18	259.67±51.95	258.28±32.66	269.85±37.86	272.18±29.01	5.07	0.697
VH : CD	7.92±0.73	8.01±1.45	7.81±1.16	8.10±0.96	8.53±1.30	0.16	0.672
Crypt area; CA (µm <sup>2</sup> )	7,956.93 ±1,101.38	7,415.02 ±1,497.62	7,531.23 ±1,790.64	7,340.22 ±2,270.67	7,871.44 ±2,663.14	265.92	0.936
MMT <sup>2</sup> (µm)	291.08±46.21	277.98±35.92	331.82±67.86	318.45±54.24	314.20±29.70	7.16	0.111
<b>Jejunum morphology</b>							
Villus height; VH (µm)	1704.73 ±91.13 <sup>b</sup>	1711.55 ±130.30 <sup>b</sup>	1703.38 ±88.80 <sup>b</sup>	1770.07 ±80.51 <sup>a,b</sup>	1816.99 ±66.56 <sup>a</sup>	14.27	0.032
Villus width; VW (µm)	214.47 ±41.06 <sup>b</sup>	256.28 ±55.28 <sup>b</sup>	215.86 ±39.86 <sup>b</sup>	224.57 ±33.19 <sup>a,b</sup>	289.60 ±72.11 <sup>a</sup>	7.97	0.006
Crypt depth; CD (µm)	294.57 ±35.76	232.39 ±38.76	252.84 ±40.36	256.96±65.04	285.46±62.04	7.51	0.051
VH : CD	5.85±0.68	7.51±1.07	6.93±1.36	7.30±1.95	6.63±1.40	0.20	0.074
Crypt area; CA (µm <sup>2</sup> )	4,597.87 ±1,365.83	5,636.79 ±673.55	5,224.38 ±2,108.18	5,694.45 ±1,643.51	5,830.50 ±2,624.77	253.31	0.553
MMT (µm)	263.30±51.89	308.46±90.43	279.67±36.37	320.16±67.29	293.82±51.33	8.91	0.279
<b>Ileum morphology</b>							
Villus height; VH (µm)	1,117.54±129.01	1,126.88±193.18	1,039.38±40.83	1,108.24±76.85	1,153.44±102.83	17.16	0.303
Villus width; VW (µm)	269.99±36.17	244.25±50.41	208.87±59.04	239.78±44.87	252.26±50.81	7.21	0.098
Crypt depth; CD (µm)	278.97±53.86	287.37±52.08	272.85±59.38	298.11±75.63	311.68±58.97	8.44	0.628
VH : CD	4.07±0.45	3.98±0.69	3.98±0.89	3.97±1.17	3.85±0.92	0.12	0.987
Crypt area; CA (µm <sup>2</sup> )	3,885.22 ±940.12	3,946.79 ±2,877.55	3,938.85 ±1,096.12	3,557.84 ±1,004.38	3,837.39 ±1,011.24	214.38	0.981
MMT (µm)	333.82±35.11	300.81±47.42	301.58±26.26	337.68±36.25	305.68±34.24	6.19	0.134

<sup>a,b</sup> Mean values with different letters in the same row indicate significant differences (*p*-value < 0.05). <sup>1</sup> CON: control diet; BS 5%: basal diet supplemented with the banana stem at 50 g/kg of diet; BS 10%: banana stem supplementation at 100 g/kg of diet; NG 5%: basal diet supplemented with the Napier grass at 50 g/kg of diet; NG 10%: basal diet supplemented with the Napier grass at 100 g/kg of diet; <sup>2</sup> MMT: Muscularis mucosae thickness; <sup>3</sup> SEM: standard error of the mean.





**Figure 1** Histological representations of the H&E-stained duodenal sections of Thai native chicken (n = 30 with 10 birds/group) receiving control diet (a), banana stem supplementation at a level 5% (b), 10% (c) of diet and Napier grass supplementation at a level 5% (d), 10% (e) of diet, respectively. Magnification was with 5x objective lens. Scale bars represent 100 µm.



**Figure 2** Histological representations of the H&E-stained jejunal sections of Thai native chicken ( $n = 30$  with 10 birds/group) receiving control diet (a), banana stem supplementation at a level 5% (b), 10% (c) of diet and Napier grass supplementation at a level 5% (d), 10% (e) of diet, respectively. Magnification was with 5x objective lens. Scale bars represent 100  $\mu\text{m}$ .

## DISCUSSION

Non-ruminants are less effective at using protein from forage sources because of the absence of appropriate microbes and enzymes in their digestive tract, which can ferment and digest fiber (Knudsen, 1997). Low digestibility causes poor growth in poultry. However, not all plants-based fibers are equal, which has varying physiological effects on birds, including, but not limited to, digestibility, growth yield and microbial fermentation (Singh and Kim, 2021). The term dietary fiber (DF) has the chemical definition describes it as “the sum of non-starch polysaccharides (NSP) and lignin” The main components of dietary fiber are included in total carbohydrates (Knudsen, 2001). In this study, different types on fiber source were analyzed and compared concerning their nutritional composition. To evaluate the difference between banana stem and Napier grass, feed fiber source supplementation was compared in terms of their productive performance in feeding crossbred native chickens. The experimental set-up followed realistic conditions in a typical small-holder production system: using the BS and NG to enhance the feed with locally available feed material.

The addition of BS to the diet of native chickens did not affect to feed intake but it affects to body weight and feed conversion ratio. Other researchers have found similar effects in poultry using fermented banana stems (Arjin et al., 2021), banana peel (Siyal et al., 2016), banana leave fermented (Mandey et al., 2015). Moreover, NG supplementation also had the same effect (Asaduzzaman, 2019; Khempaka et al., 2021; Kiggundu et al., 2022) Although the FI volume is not different, but the daily body weight gain was significantly indicated that replacing 5-10% concentrate with Napier grass had no detrimental effect on body weight gain. Rather, there was a positive correlation between concentrate and grass intake. This result could be supported by who reported that the turkey can obtain nutrients from forage because this poultry species is better able to digest fiber due to larger microbial population in their digestive tracts. Due to the inclusion of forage, the 'grass factor' has been beneficial for growth (Nixey, 2010). Forage consumption varies according to the type of forage. Poultry on pasture can consume 5 to 20% forage, depending on the age and quality of forage. In addition, this result reflects that replacement of a certain amount of concentrate by fiber give better feed efficiency with higher body weight, which happened in the group was supplemented with NG 5% and 10% of the diet. This may occur because moderate levels of fiber acted as nutrient diluents and did not affect digestion and absorption of nutrients (Hetland and Svihus, 2001). In fact, a moderate amount of fiber increased the space in the gizzard, making it easier for HCl, bile acids and enzymatic secretions to function. Thus, a better feed utilization efficiency was shown.

The functionality of the gastrointestinal tract of birds is affected by diet. Internal organ weights were relatively higher for birds with a supplementation NG 5%. In poultry, proventriculus is known as the gastric compartment of the gastrointestinal tract (GIT) (Svihus, 2014). Birds in the control group had a proventriculus significantly lighter than the substitution rates of the high fiber sources, possibly because these organs are involved in the digestion of the fibers in poultry. This finding is consistent with previous reports that birds fed high-fiber diets had significantly longer and wider gastrointestinal tracts compared to traditional low-fiber birds (Jørgensen et al., 1996; Mpofu et al., 2016). Meanwhile, studies have revealed that the kind and make-up of the food have an impact on the mean retention period and rate of feed passage in the GIT of birds (Svihus, 2014). In addition, the Pradu Hang dum chickens in the current study also produced more thigh yield than previously had been reported (Jaturasitha et al., 2008). The production of the Thai indigenous chicken's legs and wings was higher than that of other chickens (Tang et al., 2009; Wang et al., 2009; Zhao et al., 2009). This can be explained by the properties of muscle fiber, such as their composition and morphological characteristics, as the property of muscle fiber as the main component of skeletal muscle is one of the key elements influencing BW and skeletal muscle mass (Picard et al., 2006; Koomkrong et al., 2015).

The most practical and affordable approaches to safeguard poultry from heat stress (HS) are nutritional solutions. These remedies entail either choosing nutrients with modest heat increments or supplying bioactive nutrients that enhance the physiological dysfunctions linked to HS (Shakeri and Le, 2022). Research is therefore required to determine the appropriate nutrients and their perfect forms that enhance the performance of chickens exposed to HS. Meanwhile, MDA and GSH level can reflect the oxidative status in the body (Liu et al., 2011). MDA is a biomarker of oxidant status because it is the end

product of lipoperoxidation (Pérez et al., 2012). Also, GSH is the natural antioxidant in the body. So, MDA and GSH level were used to determined antioxidant efficiency in this experiment. These studies have shown that BS 5% and NG 10% improve MDA levels and increase GSH in serum. Bananas and Napier grass have antioxidant qualities, according to earlier reports. For the extracts from leaf stalks, banana stem and ripe banana peels, there was total phenolic content in the range of 9.42 - 11.27 mgGAE/g and more prevalent in *Musa Cavendish* peel (907 mg/100 g dry weight.) than in pulp (232 mg/100 g dry weight.) (Someya et al., 2002), according to the total phenolic content of Napier grass in the leaves and stems of Napier grass was 4.29 g/100 g fresh weight (Song et al., 2010), as previously reported. From reported of Yao et al. (2010) that polyphenols content was positively correlated with antioxidant enzyme activity. Therefore, the increase in GSH-Px enzyme activity in the chicken of this experiment. This may be due to the content of phenolic polyphenols found in BS and NG that stimulated GSH-Px enzyme activity and antioxidant capacity in animals and meat. The mechanism of activation is caused by polyphenols to activate the antioxidant response element (ARE) of the gene group glutathione-related enzymes, namely GSH-Px glutathione reductase and glutathione S-transferase genes (Shih et al., 2007). Also, polyphenols have been investigated as food additives for livestock, as natural antioxidants, they are more acceptable by the market as they are considered safe for human health. Based on their special properties, they may have anti-inflammatory effects due to their unique features, and they can increase feed intake and growth rate (Serra et al., 2021).

The impact of fiber on the intestinal histomorphology status varies. Chickens fed viscous components like citrus pectin and xanthan gum had shorter villi, but insoluble fiber was shown to encourage villi formation (Montagne et al., 2003; Bederska-Łojewska et al., 2017). Fibers can have an impact on the digestive system by raising the height of the villi and the depth of the crypts (Dahlke et al., 2003). It is demonstrated that adding 10% fiber to diet for 2 weeks increase cell proliferation in the villi of gastro intestinal tract, Arjin et al. (2021) reported that crossbred pigs receiving a low-protein diet supplement with fermented banana stem had increased villi height and villi width, which increased nutrient absorption, also adding cellulose to the low fiber diets increase DNA synthesis and cell multiplication in gastro intestinal tract (Jin et al., 1994; Klurfeld, 1999). The architecture of the intestinal mucosa can provide important details on how the intestines function. Villi that are taller signify that there is more surface area for better absorption of nutrients that are accessible (Awad et al., 2008), an increase in digestion and absorption is linked to an increase in the villi to crypt ratio (Montagne et al., 2003). In the past, the addition of lignocellulose at 2%, helped to reduce the amount of *Clostridium* spp. A 2% dietary addition of lignocellulose had no impact on the counts of *Bifidobacterium* spp., *Bacteroides*, *Bacillus* spp., or *Lactobacillus* spp. (Kheravii et al., 2017). Additional, giving probiotics from *Kumpai Tembaga* grass silage into the ration at a dose of 0.2% was able to increase the percentage of small intestinal (Sandi et al., 2021). Some research revealed that fiber functions as prebiotics in the large intestine and ceca and may help the chicks' gastrointestinal system choose a healthy microbiome (Teng and Kim, 2018).

## CONCLUSIONS

The findings of this study showed that including a fiber source in the diet had positive benefits on growth performances (5% of BS and 10% of NG in diets), carcass, intestinal health, and lower blood MDA levels. As a result, it is advised that this supplement be administered as a fiber source in Thai native chicken to assist lower costs.

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## AUTHOR CONTRIBUTIONS

Patcharee Kanjak: investigation, methodology, formal analysis, writing original draft; Chompunut Lumsangkul and Wipasiri Chaiphun: supervision; Wanaporn Tapingkae: project administration; Tossapol Moonmanee, Suwit Chotinun and Mongkol Yachai: writing review and editing; Montri Punyatong: validation, conceptualization, writing review and editing. All authors have read and agreed to the published version of the manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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