Research Article

Dietary of Synbiotic Derived from Trimmed Asparagus by Products Combined with Probiotic Supplementation on Digestibility, Gut Ecology, Intestinal Morphology and Performance of Broilers

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Abstract | Synbiotics are made by combining prebiotics and probiotics to improve gut microbiology, digestibility, and performance of broilers. This experiment was carried out to investigate the effects of synbiotic from trimmed asparagus by-products (TABP) combined with probiotic supplementation in broiler diets on the apparent nutrient digestibility, cecal microbiota, small intestinal morphology, and performance. Three hundred twenty-one days old, Ross 308® chicks; were raised under ambient temperature and assigned to a completely randomized design with four treatments and four replications per treatment (n = 20). The treatment consisted of a control diet based on a corn-soybean basal diet and a control diet supplemented with 10, 30, and 50 g/kg TABP combined with 2 g/kg probiotics. This study shows that TABP combined with probiotic supplementation significantly affected the apparent digestibility of dry matter, ether extract, crude fiber, and crude protein (p<0.05). The supplementation of TABP combined with probiotics increased the lactic acid bacteria, *Enterococcus* sp., and volatile fatty acids (p < 0.05) and decreased *Salmonella* spp. and *Escherichia coli* in the cecum of different treatment to control groups (p < 0.05). In addition, TABP combined with probiotics supplementation increased the villus height, villus surface area, and the depth of the crypt of Lieberkühn of the duodenum, jejunum, and ileum (p < 0.01). Moreover, TABP combined with probiotic supplementation significantly affected the feed intake and average daily gain of broilers (p < 0.05). The data showed that minimum supplementation of synbiotics from TABP at 10 g/kg in combination with probiotics (2 g/kg) in diets has the potential to improve digestibility, gut ecology, and productivity of broilers.

Keywords | Asparagus, Broiler, By-product, Feed additive, Synbiotics

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INTRODUCTION

Agricultural waste contributes to pollution issues as a result of ineffective management. Many countries invest in research and development to benefit from agricultural waste under the bio, circular, and green economy concepts. Asparagus has received increasing popularity from consumers due to its distinct flavor, low calories, and presence of beneficial phytochemicals. Asparagus is a great source of prebiotics. Fructans and

dietary fiber are responsible for the healthy properties of asparagus spear consumption (Majumder et al., 2017). During the processing, the asparagus spears are trimmed for the quality parts, which is accounted for 60%-70% of the whole plant. The trimmed asparagus by-products (TABP) and unqualified asparagus are discarded in general practices. The edible portion of asparagus has fructans approximately 0.5%-2% (dry weight) depending on the variety. Furthermore, fructans reserve polysaccharides that accumulate in the roots of the asparagus and account for approximately 25% of the fresh weight (Singh and Singh, 2010; Viera-Alcaide et al., 2020). Previous research has shown that asparagus extract has a positive effect on the growth of lactic acid bacteria (Majumder et al., 2017). It has been demonstrated that asparagus promotes the growth of beneficial bacteria.

Poultry production with food safety was a difficult issue for industrial production systems that prioritize productivity and yield. After the European Union announced the elimination of antibiotics in animal feed in 2006 and the United States announced a voluntary plan to phase out antibiotics in feed in 2013 (Wang et al., 2016). However, the appearance of antibiotic resistance in pathogens identified as public health risks has resulted in the reduction or prohibition of routine antibiotic supplementation for agricultural use in several parts of the world (Ricke et al., 2020). The ultimate goal of broiler diet development is improved nutrient digestibility, increased feed utilization, reduced feed costs, and minimized nutrient release into the environment. Nutribiotics such as probiotics, prebiotics, phytobiotics, and synbiotics are alternative feed additives that are used to solve such problems. Synbiotics are created by combining prebiotics and probiotics as they have a synergistic effect to improve productive performance, promote the activity of beneficial gut micro bacteria, and decrease ammonia release (Audrey et al., 2020). Prebiotics are food components that selectively stimulate the growth and activity of probiotics. They are non-digestible food ingredients composed of short-chain carbohydrates, primarily oligosaccharides such as fructooligosaccharides (FOS), and inulin (Sugiharto, 2016). Probiotics are live, nonpathogenic microorganisms including yeast, *Bacillus* sp., *Lactobacillus* sp., *Bifidobacterium*, and others (Bai et al., 2017). The lower gastrointestinal tract is a community of microorganisms that play an important role in the host's gut health and digestion efficiency. Thus, microbial balance in the gastrointestinal tract affected the efficient fermentation process in the lower gut. It affects the formation of short-chain fatty acids, which promote the development of intestinal epithelial cells for increased absorption and bird growth. Hutsko et al. (2016) described the ability of synbiotics to improve the ecological balance of the gut. In addition, synbiotics provide numerous benefits, including inhibiting of colonization of pathogens (Wang et al., 2016), enhancing nutrient digestibility (Palamidi et al., 2016), improving gut integrity, immune function, and strengthening intestinal absorption cells (Hu et al., 2022), promoting health, and enhancing growth performance (Mohammed et al., 2018).

According to the previous study of Nopparatmaitree et al. (2022), TABP supplementation can improve intestinal ecology, productivity, and provide chicken meat with beneficial properties. However, no research has been conducted to describe synbiotics derived from the use of asparagus by-products in broiler diets with beneficial microorganisms. Therefore, additional research is required to assist in guiding the recycling of asparagus waste for use as synbiotics in bird diets. The main idea of this research will lead to an explanation of the synergism of synbiotics from the combination of prebiotics from TABP and probiotics as feed additives for the improvement of feed utilization efficiency, gut ecology, and broiler productivity as well as the addition of value from agricultural waste and biological base for maximum benefit. This experiment was conducted to determine the effects of synbiotic supplementation from TABP combined with probiotics in broiler diets on the digestibility, cecal microorganisms, small-intestine histomorphology, productive performance and economic benefit return.

MATERIALS AND METHODS

Ethical approval

The Animal Care Protocol Management and Review Committee of Silpakorn University's Faculty of Animal Science and Agricultural Technology approved the protocol for this study (record no. ASAT SU0101/2562).

Experimental product

TABP were collected from the Royal Project of His Majesty the King of Thailand, Hup-krapong, Cha Am district, Phetchaburi province. TABP were sliced and oven-dried at 60°C for three days. Then, dried TABP was ground by pulverizing machine (RT-34, Chyuntseh industrial CO., LTD.) to achieve uniform granules of 2 mm. The chemical composition of TABP was analyzed according to AOAC (2005), as well as FOS content was determined using thin-layer chromatography (TLC) according to Reiffová and Nemcová's (2006). The results of TABP nutrient composition are as followed; 86.80% dry matter (DM), 18.50% crude protein (CP), 0.61% ether extract (EE), 37.62% crude fiber (CF), 90.77% organic matter (OM), 2175.23 kcal/kg gross energy (GE), 0.10% calcium, 0.66% phosphorus and 1.84% FOS. The probiotics used in this trial contained of *Lactobacillus acidophilus* (10 Log cfu/g), *Lactobacillus plantarum* (10 Log cfu/g), *Pediococcus pentosaceus* (10 Log cfu/g), *Streptococcus faecium* (10 Log

cfu/g), *Saccharomyces cerevisiae* (10 Log cfu/g), *Bacillus subtilis* (10 Log cfu/g), and *Bacillus licheniformis* (10 Log cfu/g) and an added carrier to complete 1 kg.

Experimental design, birds, and diets

In a completely randomized design with four treatments and four replications, with 20 birds per experimental unit, 320 one-day-old Ross 308® chicks (160 male and 160 female) were distributed. The chicks were vaccinated against Marek's disease in the hatchery, but vaccinations against infectious bronchitis and Newcastle were given at 7 and 14 days. The following are the treatments:

- Treatment 1 = Control diet without supplementation (base diet)
- Treatment 2 = Control diet + Supplementation of 10 g/kg of TABP combine with 2 g/kg probiotics
- Treatment 3 = Control diet + Supplementation of 30 g/kg of TABP combine with 2 g/kg probiotics
- Treatment 4 = Control diet + Supplementation of 50 g/kg of TABP combine with 2 g/kg probiotics

The birds were fed ad libitum drinking water, as well as a starter (0–21 days) and finisher (22–35 days) diets designed in accordance with the recommendations of the National Research Council (1994) (Table 1).

Nutrient digestibility

Another 32 birds (several days old) were placed in 16 cages with wired bottoms (two birds per cage). The indicator method was used to assess the nutrient digestibility in broilers over two periods, namely, adaptation (0–18 days) and collection periods (19–21 days), following the methoddescribed by Fenton and Fenton (1979).

Table 1: Ingredient composition and nutritive value of experimental diet.

Experimental diet*	Starter diet (0-21 day)				Finisher diet (22-35 day)			
	T1	T2	T3	T4	T1	T2	T3	T4
Ingredient composition (%)								
Corn	49.50	48.50	49.50	49.50	46.92	46.92	46.92	46.92
Soybean meal(44%CP)	36.50	36.32	35.96	35.60	30.90	30.72	30.36	30.00
TABP[1]	-	1.00	3.00	5.00	-	1.00	3.00	5.00
Probiotics[2]	-	0.20	0.20	0.20	-	0.20	0.20	0.20
Defatted rice bran	8.00	7.18	5.54	3.90	12.50	11.68	10.04	8.4
Rice bran oil	1.70	1.70	1.70	1.70	5.56	5.56	5.56	5.56
Limestone ($CaCO_3$)	1.35	1.35	1.35	1.35	1.20	1.20	1.20	1.20
DCP (18%P)	2.10	2.10	2.10	2.10	1.90	1.90	1.90	1.90
Choline Chloride-L	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
NaCl	0.14	0.14	0.14	0.14	0.23	0.23	0.23	0.23
DL-Methionine (99%)	0.34	0.34	0.34	0.34	0.28	0.28	0.28	0.28
L-lysine (98.5%)	-	-	-	-	0.22	0.22	0.22	0.22
L-Threonine (98.5%)	0.15	0.15	0.15	0.15	0.06	0.06	0.06	0.06
Premix[3]	0.20	0.20	0.20	0.20	0.02	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutritive value from laboratory analysis (%)								
Dry matter	90.35	90.31	91.02	90.67	91.13	90.76	90.87	90.49
Crude protein	23.56	23.91	23.71	23.57	20.49	20.36	20.41	20.33
Ether extract	5.39	5.23	5.60	5.00	5.51	5.30	5.52	5.63
Crude fiber	4.26	4.19	4.31	4.49	3.54	3.79	4.87	4.90
Ash	8.11	7.93	7.36	6.35	6.82	7.10	6.61	7.31
Metabilizable energy (Kcal/kg)	4,079.30	4,033.60	4,012.30	4,073.90	4,089.60	4,069.90	4,148.90	4,082.00

*T1: ration without TABP, T2: ration + 10 g/kg TABP powder; T3: ration + 30 g/kg TABP powder, and T4: ration + 50 g/kg TABP powder. [1]TABP = Trimmed asparagus by-products; [2]Probiotics used in this trial contained of *Lactobacillus acidophilus* (10 Log cfu/g), *Lactobacillus plantarum* (10 Log cfu/g), *Pediococcus pentosaceus* (10 Log cfu/g), *Streptococcus faecium* (10 Log cfu/g), *Saccharomyces cerevisiae* (10 Log cfu/g), *Bacillus subtilis* (10 Log cfu/g), and *Bacillus licheniformis* (10 Log cfu/g) and an added carrier to complete 1 kg; [3]Each one kilogram of vitamin–mineral premix contained 20.02 MIU of retinal palmitate, 9.10 MIU of cholecalciferol, 136.50 g of DL-3-tocophyryl acetate, 5.46 g of phylloquinone, 5.46 g of thiamine, 14.56 g of riboflavin, 27.30 g of Ca-D-pantothenate, 7.28 g of pyridoxine, 109.20 g of niacin, 3.64 g of folic acid, 29.12 mg of cobalamin, 237.00 mg of D-biotin, 120 g of manganese, 3.00 g of selenium, 1,000 mg of zinc, 160.00 mg of copper, 400.00 mg of ferrous, 12.50 g of iodine.

The diets were supplemented with chromic oxide at a rate of 3 g/kg diet as an analytical marker for measurement. The birds were fed with a chromic oxide diet for 0–21 days, and their feces were collected in 3% H_2SO_4 in a plastic bag and stored at −20°C until the time of analysis for nutrient retention, as described by Mountzouris et al. (2010), to determine the nutrient digestibility; the feed and feces were dried at 60°C and then analyzed (AOAC, 2005). Standard methods were used to determine the DM, OM, CF, EE, CP, and GE of feed and feces samples in accordance with AOAC (2005). The chromic oxide content was determined using a spectrophotometer and standard absorption curves of chromic oxide at various levels following the method described by AOAC (2005). The nutritional value and chromic oxide content were used to calculate the apparent dry matter digestibility (ADMD) and apparent nutrient digestibility (AND) using the method described by Zewdie (2019), and the following formulas were used:

1.) ADMD = [{(% Cr_2O_3 in feces − % Cr_2O_3 in diets) × 100}/{% Cr_2O_3 in feces}]

2.) AND = 100 − [100 × {(% Cr_2O_3 in diets × % Cr_2O_3 in feces) / (% nutrient in feces × % nutrient in diets)}]

Cecal microbiota, volatile fatty acid, and small intestine morphology

Broilers were restricted feed for at least 6 h before their slaughter at 21 days old. Four broilers were slaughtered in each experimental unit (two males and two females) to collect cecum content samples to count the number of microorganisms and a small-intestine sample from the duodenum, jejunum, and ilium section in accordance with the method described by Yang et al. (2016). Small-intestine samples were collected in a 10% formaldehyde solution using the method described by Gava et al. (2015) for analysis. In the analysis of laboratory samples, microorganisms were measured using the culture technique lactic acid bacteria (LAB) (*Lactobacillus* + *Bifidobacterium* spp.), *Enterococcus* sp., *E. coli*, and *Salmonella* spp., in accordance with the method described by McDonald et al. (1983), Horn et al. (1996), and Schillinger and Holzapfel (2003). The number of microorganisms was then transformed with the base 10 log algorithm following the method described by Cengiz et al. (2015).

The volatile cyanide analysis was performed by collecting a 1.5 g sterile liquid sample in sterile water (1:1 g/v) in a screw-capped tube and storing the sample at −20°C for the analysis using the method described by Khattak et al. (2018). After homogenization and centrifugation, a 1 mL supernatant sample was packed into an ampulla with 0.2% meta-phosphoric acid and homogenized before being placed in an ice bucket for 30 min. The supernatant was extracted after centrifuging the sample at 10844 ×g for 10 min to determine the short-chain fatty acid content using the method described by Wang et al. (2005). The sample solution was analyzed for short-chain fatty acid content using gas chromatography (HP 5890 Series II GC; Agilent J&W 30 m 0.535 mm 1.00 micron HP-FFAP column technique), and a flame-ionization detector was used as a measure. The sample injected with 4-methylvaleric acid (Alfa Aesar, United Kingdom) served as an internal standard for acetic acid, propionic acid, butyric acid, and lactic acid analysis. The composition of VFAs was determined by comparing them to standard solutions using the method described by Khattak et al. (2018).

The small-intestine samples were embedded in paraffin and biopsied to a thickness of 5 µm to prepare the sample slides for histology. Alcian blue was used to stain the biopsy, which was then mounted on the slide following the method described by Shang et al. (2015). The small-intestine histology was measured with an Olympus BX 50, 20 × optical magnification optical microscopy and analyzed with the Motic Images 2.0 Multi language program (Tsirtsikos et al., 2012). Ten villi were sampled per slide to determine the villus height, villus width, and cryptal depth using the method described by Shang et al. (2015), whereas the villus surface area was calculated from 2π × (average villus width/2) ×villus height (Sakamoto et al., 2000).

Productive performance and economic benefit return

The broilers were raised for 35 days in total, and the raising period was divided into two: 0–21 and 22–35 days. The total feed intake and initial and final weight of broilers were recorded to calculate average daily feed intake (ADFI) and average daily gain (ADG) for each growth period and feed conversion ratio (FCR). Daily mortality was recorded, and the percentage of viability was calculated using the data obtained. For the analysis of productive performance indicators, such as body weight gain (BWG), ADG, ADFI, FCR, and viability, the following formulas were used: BWG (g on period) = BW (g) at the end period − BW (g) on the first day; ADG (g/chick/day) = BWG/days of growth period; ADFI (g/chick/day) = total feed intake/days of growth period; FCR (feed/gain) = cumulative feed intake (kg)/total weight gain (kg); viability (%) = chicks remaining at the end of period (%), in accordance with the method described by Marcu et al. (2013); productive index (PI) was calculated using the formula PI = (daily weight gain (kg) × livability/FCR) × 100, following the method described by Barbosa et al. (2017). In addition, economic benefit return indicators, such as feed cost per gain (FCG), salable bird return (SBR), net profits return per bird (NPR), and return of investment (ROI), were calculated using the following formula: FCG = (FCR × feed cost × BWG), SBR = (the price of live chicken (40 THB) × BW),

NPR = (SBR – FCG), and ROI = (NPR/FCG) × 100 (El-Aziz et al., 2019).

Statistical analyses

The experimental data were used in the analysis of variance (ANOVA) for a completely randomized design. When the ANOVA showed significant differences using the method described by Steel and Torrie (1992), Tukey's honestly significant test was applied in accordance with the R Core Team's description of R program version 3.5.1 (R Core Team, 2018). The following statistical model was used for the experiment: $Y_{ij} = \mu + T_i + e_{ij}$ where μ = general mean, T_i = effect of treatment (i = control and supplementation of TABP at 10, 30, and 50 g/kg with probiotics 2 g/kg), and e_{ij} = random error associated with Y_{ij} observation.

RESULTS AND DISCUSSION

Apparent nutrient digestibility

Broilers fed with diets containing 10, 30, and 50 g/kg TABP supplements with 2 g/kg probiotics had higher digestibility of DM, CP, EE, and CF than the control group ($p < 0.05$). In broiler diets, the increase in TABP supplementation (10, 30, and 50 g/kg) with 2 g/kg probiotics resulted in a linear increase in DM digestibility ($p < 0.05$) and a quadratic increase in CP, EE, and CF digestibility ($p < 0.05$) (Table 2).

Cecal microbiota and volatile fatty acid

Broilers fed with 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics had higher levels of LAB and *Enterococcus* sp. in the cecum than the control group ($p < 0.01$). Supplementing diets with 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics containing LAB in cecum resulted in a quadratic increase ($p < 0.01$), whereas the presence of *Enterococcus* sp. in the cecum resulted in a linear increase ($p < 0.01$). Additionally, broilers fed with 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics had lower levels of *Salmonella* spp. and *E. coli* in the cecum than the control group ($p < 0.01$). As shown in Table 3, the supplement diets with 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics containing *Salmonella* spp. and *E. coli* in cecum resulted in a quadratic decrease ($p < 0.01$). Furthermore, this study showed that broilers fed with diets containing 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics had higher levels of VFA, acetic acid, propionic acid, and butyric acid in the gut than control broilers ($p < 0.01$) and showed a quadratic increase in these volatile fatty acids ($p < 0.01$).

Table 2: Effects of TABP and probiotics supplementation in broiler diets on apparent nutrient digestibility.

Apparent nutrient digestibility (%)	Control	Level of TABP with 0.2% probiotics supplementation in diets (g/kg)			SEM	P-value	Trend analysis
		10	30	50			
Dry matter	83.77^{c}	85.72^{ab}	84.88^{b}	87.31^{a}	0.49	0.02	L
Crude protein	80.44^{c}	89.48^{a}	87.04^{ab}	86.10^{b}	1.02	0.02	Q2
Ether extract	92.28^{c}	94.13^{ab}	95.06^{a}	93.88^{b}	1.74	0.03	Q2
Organic matter	86.78	88.13	86.99	86.10	0.63	0.52	NS
Gross energy	87.44	89.48	90.04	89.10	0.63	0.06	NS
Crude fiber	77.89^{c}	87.36^{ab}	90.55^{a}	87.83^{ab}	0.27	0.01	Q2

^{a,b} Mean with symbol with in same row differ significantly different ($P<0.05$); SEM: Standard error of mean, NS: Not significantly different ($P>0.05$); L: Linear.

Table 3: Effects of TABP and probiotics supplementation in broiler diets on cecal microbiota and volatile fatty acids content.

Cecal microbiology and volatile fatty acids	Control	Level of TABP with 0.2% probiotics supplementation in diets (g/kg)			SEM	P-value	Trend analysis
		10	30	50			
Cecal microbiology (Log $_{10}$ CFU/ml)							
Lactic acid bacteria*	11.35^{B}	12.10^{A}	12.00^{A}	12.08^{A}	0.16	<0.01	Q2
Enterococci sp.	6.70^{C}	7.01^{B}	7.18^{AB}	7.44^{A}	0.13	<0.01	L
E. coli.	8.17^{A}	7.57^{B}	7.34^{BC}	7.15^{C}	0.11	<0.01	Q2
Samonella spp.	3.73^{A}	3.26^{B}	3.19^{B}	3.20^{B}	0.09	<0.01	Q2
Volatile fatty acids: VFA (μmol/ml)							
Total VFA	71.22^{B}	88.97^{A}	87.26^{A}	84.76^{A}	4.30	<0.01	Q2
Acetic acid	48.64^{B}	55.83^{A}	56.27^{A}	54.02^{A}	2.88	<0.01	Q2
Propionic aid	9.05^{B}	10.43^{A}	10.48^{A}	10.50^{A}	0.37	<0.01	Q2
Butyric acid	9.50^{B}	10.86^{A}	10.91^{A}	10.95^{A}	0.38	<0.01	Q2

* *Lactobacillus* and *Bifidobacteria*; ^{A,B} Mean with symbol with in same row differ significantly different ($P<0.01$); SEM: Standard error of mean; NS: Not significantly different ($P>0.05$); L: Linear; Q2: Quadratic.

Small-intestine histomorphology

The supplementation of 10 and 30 g/kg TABP in combination with 2 g/kg probiotics in broiler diet increased the duodenum villus height ($p < 0.01$). Nonetheless, the increased TABP supplementation in combination with 2 g/kg probiotics resulted in a positive quadratic trend ($p < 0.01$) in duodenal villus height, villus surface area, and cryptal depth ($p < 0.01$). Furthermore, broilers fed with diets containing 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics had increased villus height, villus width, villus surface area, and cryptal depth of the jejunum ($p < 0.01$). The increase of jejunum histomorphology values showed a positive quadratic trend corresponding to levels of TABP supplementation ($p < 0.01$). This study also revealed that broilers that received 10 and 30 g/kg TABP in combination with 2 g/kg total probiotics had higher villus height, surface area of the villus, and crypt of Lieberkühn depth of ileum than the control broilers ($p < 0.01$). Supplementing diets with 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics resulted in a quadratic increase of the villus height and crypt of Lieberkühn depth of ileum ($p < 0.01$), whereas the villus surface area of ileum showed a cubic increase ($p < 0.01$) (Table 4).

Productive performance and economic benefit return

This study discovered that broilers fed with diets containing 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics during the 22–35- and 0–35-day periods had higher ADFI, and ADG than broilers in the control group ($p < 0.05$). In addition, during the 22–35- and 0–35-day periods, ADFI increased linearly ($p < 0.05$) in response to the supplementation of 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics. The ADG increased quadratically ($p < 0.05$) in response to the supplementation of 10, 30, and 50 g/kg TABP in combination with 2 g/kg probiotics. The data in Table 5 show no significant effects on viability rate and PI among treatment groups across all rearing periods ($p > 0.05$). The level of TABP with 2 g/kg probiotics in broiler diets had no effect on any of the economic benefit return indicators, including FCG, SBR, NPR, and ROI ($p > 0.05$).

This study demonstrated the efficacy of synbiotics derived from TABP supplementation with 2 g/kg total probiotics in broiler diet on positively contributes to the improvement of feed utilization efficiency by increasing digestibility, improving intestinal ecology, and developing intestinal morphology, which results in improved broiler growth performance. Furthermore, broilers fed TABP synbiotics plus 2 g/kg total probiotics in broiler diets had higher digestibility of DM, CP, EE, and CF than control group broilers. A scientifically credible reason has been linked to explaining these results from a report by Huang et al. (2015), who described the constituents of high levels of FOS and inulin in asparagus. These structures are classified as prebiotics because they must

Table 4: Effects of TABP and probiotics supplementation in broiler diets on small intestinal histomorphology.

Small intestinal histomorphology	Control	Level of TABP with 0.2% probiotics supplementation in diets (g/kg)			SEM	P-value	Trend analysis
		10	30	50			
Duodenum							
Villus height (mm)	1.42^{C}	1.64^{AB}	1.75^{A}	1.52^{BC}	0.08	<0.01	Q2
Villus wide (mm)	0.14	0.13	0.14	0.15	0.01	0.16	NS
VSA (mm^2)	0.61^{B}	0.71^{AB}	0.79^{A}	0.71^{AB}	0.04	<0.01	Q2
Cryptal depth (mm)	0.22^{B}	0.24^{AB}	0.25^{A}	0.23^{AB}	0.01	<0.01	Q2
VH: CD	6.56	6.93	6.99	6.64	0.46	0.47	NS
Jejunum							
Villus height (mm)	1.11^{B}	1.31^{A}	1.27^{A}	1.27^{A}	0.05	<0.01	Q2
Villus wide (mm)	0.14^{B}	0.17^{A}	0.17^{A}	0.17^{A}	0.01	<0.01	Q2
VSA (mm^2)	0.47^{B}	0.69^{A}	0.68^{A}	0.66^{A}	0.06	<0.01	Q2
Cryptal depth (mm)	0.21^{B}	0.24^{A}	0.25^{A}	0.25^{A}	0.01	<0.01	Q2
VH: CD	5.29	5.50	5.07	5.17	0.29	0.24	NS
Ileum							
Villus height (mm)	0.76^{C}	0.84^{AB}	0.86^{A}	0.82^{B}	0.02	<0.01	Q2
Villus wide (mm)	0.13	0.14	0.14	0.13	0.01	0.97	NS
VSA (mm^2)	0.24^{B}	0.44^{A}	0.37^{A}	0.34^{AB}	0.05	<0.01	C
Cryptal depth (mm)	0.15^{B}	0.17^{A}	0.17^{A}	0.17^{A}	0.01	<0.01	Q2
VH: CD	5.29	5.02	5.12	4.99	0.20	0.24	NS

^{A,B} Mean with symbol with in same row differ significantly different ($P<0.05$); SEM: Standard error of mean; NS: Not significantly different ($P>0.05$); L: Linear; Q2: Quadratic; C: Cubic; VSA: Villus surface area; VH:CD = Villus height: Cryptal depth.

Table 5: Effects of TABP and probiotics supplementation in broiler diets on productive performance and economic benefit return.

Performance and economic benefit return	Control	Level of TABP with 0.2% probiotics supplementation in diets (g/kg)			SEM	P-value	Trend analysis
		10	30	50			
Average daily feed intake (g/bird/day)							
0-21 day	61.68	59.84	62.10	65.19	0.61	0.08	NS
22-35 day	179.72^{b}	200.57^{a}	205.69^{a}	208.07^{a}	2.91	0.03	L
0-35 day	108.89^{b}	116.13^{ab}	119.54^{a}	122.34^{a}	1.43	0.04	L
Average daily gain (g/bird/day)							
0-21 day	41.30	40.18	40.63	39.22	0.49	0.54	NS
22-35 day	77.61^{b}	94.41^{a}	91.54^{a}	89.77^{a}	1.41	0.01	Q2
0-35 day	55.82^{b}	61.87^{a}	60.99^{a}	59.44^{ab}	0.58	0.02	Q2
Feed conversion ratio (Feed/Gain)							
0-21 day	1.49^{B}	1.49^{B}	1.53^{B}	1.66^{A}	0.01	<0.01	Q2
22-35 day	2.33	2.13	2.25	2.32	0.05	0.56	NS
0-35 day	1.95	1.88	1.96	2.06	0.03	0.20	NS
Viability (%)							
0-21 day	98.33	98.33	100.00	98.33	0.72	0.81	NS
22-35 day	91.23	98.25	92.98	94.64	1.70	0.54	NS
0-35 day	89.47	96.49	92.98	92.98	1.52	0.49	NS
Productive index							
0-21 day	271.47	265.30	265.79	232.16	3.42	0.07	NS
22-35 day	204.43	292.43	254.64	244.83	10.64	0.10	NS
0-35 day	256.60	318.63	290.56	268.58	8.35	0.12	NS
Economic benefit return*							
FCG (USD)	2.14	2.19	2.32	2.35	0.90	0.09	NS
SBR (USD)	2.26	2.41	2.44	2.35	0.71	0.09	NS
NPR (USD)	0.12	0.21	0.12	0.01	0.98	0.20	NS
ROI (%)	5.88	9.78	5.25	0.19	1.45	0.22	NS

1 USD=31.41 THB; *FCG: Feed cost per gain; SBR: Salable bird return; NRP: Net profits return per bird and ROI: Return of investment. ^{a,b} Mean with symbol with in same row differ significantly different (P<0.05), SEM: Standard error of mean; NS: Not significantly different (P>0.05); L: Linear; Q2: Quadratic.

be neither hydrolyzed or absorbed in the superior part of gut and thus serve as selective precursors for supports growth and/or metabolic activity of members of the gut microbial community that could be considered beneficial of broilers (Guaragnia et al., 2020) as well as stimulates luminal or other systemic physiological responses that are beneficial to broilers (Ricke et al., 2020). When prebiotics (FOS and inulin in asparagus) enter the lower gut of broilers, fermentation influences the proliferation and survival of beneficial bacteria in the gut while also promoting probiotic growth (Terpou et al., 2019); two types of LAB (*Bifidobacterium* and *Lactobacillus*) produce bacteriocin, which inhibits the growth of pathogenic bacteria (Alavi et al., 2012). According to Al-Khalaifa et al. (2019), probiotics are live microorganisms, such as *Lactobacillus, Bifidobacterium*, and yeast that benefit the improvement of the intestinal microbial balance and contributes to the increased digestion of FOS and inulin prebiotics in monogastric animals; digestive enzymes in the primary gastrointestinal tract cannot digest probiotics (Davani-Davari et al, 2019). Probiotics specifically increase the digestibility of carbohydrates and fibers in cecum, which contains a large number of microorganisms and is where the primary fermentation process in chicken occurs (Józefiak et al., 2008). The increased nutritional digestibility of broiler chickens in this trial provides clear data on the benefits of using synbiotics with a combination of probiotics and prebiotics. This trial's findings are consistent with those of previous studies on the use of synbiotics, prebiotics, and probiotics, all of which play an important role in improving nutrient digestibility in broiler diets (Yun et al., 2017). Apata (2008) observed that probiotic-treated broilers had higher DM digestibility compared with the controls. In addition, Huang et al. (2005) claimed that supplementing

with prebiotics and probiotics improves nutrient digestion and absorption, which can improve chicken performance. Meng et al. (2010) demonstrated that supplementing prebiotics derived from oligosaccharides increased the digestibility of DM and CP in broiler diets while also increasing apparent metabolizable energy corrected for nitrogen (AMEn) values (Al-Sagan et al., 2017). Despite the fact that TABP supplementation at 10-30 g/kg showed good nutrient digestibility. However, increasing TABP supplementation (10, 30, and 50 g/kg) with 2 g/kg probiotics resulted in a quadratic increase in CP, EE, and CF digestibility, which could be attributed to higher fiber levels in the diet resulting in decreased digestibility. Possible explanations include insoluble fibers making up a large portion of endosperm cell walls, preventing digestive enzymes from accessing nutrients within the cell. Soluble fibers, on the other hand, tend to cause viscous conditions in the digestive tract, which can impair digestion and nutrient absorption (Jha and Mishra, 2021).

This study discovered that TABP combined with probiotics can increase the VFA production and number of beneficial microorganisms, such as LAB and *Enterococcus* sp., in the cecum of broilers; while decreasing the number of *Salmonella* spp. and *E. coli,* when compared to the control group. Józefiak et al. (2008) reported that prebiotics, such as beta-glucan and inulin, can increase LAB. However, the function of probiotics or beneficial microorganisms in the large intestine plays an important role in the entire fermentation process of FOS and inulin, producing gases, lactic acid, and short-chain fatty acids (acetic acid, propionic acid, and butyric acid), which are the result of oligosaccharide fermentation of microorganisms in the pathway bottom food (Nabizadeh, 2012). Furthermore, *Bifidobacterium* and *Lactobacillus* can produce organic acids, such as lactic acid and acetic acid, and this phenomenon may inhibit several pathogenic bacteria, such as *Salmonella* spp. and *E. coli*, and reduce colonization in the gastrointestinal tract (Kridtayopas et al., 2019). These short-chain VFAs are necessary for the physiological processes of the intestinal microflora and are beneficial for improving gut health and modulating microbial ecology (Silva et al., 2020). They cause an acidic environment that is unsuitable for the growth and division of harmful bacteria, and the increases in fermentation activity and VFA content have been linked to increased acidity, which inhibits pathogenic effects and increases nutrient digestibility (Krysiak et al., 2021). Buclaw (2016) report described the ability of *Bifidobacterium* and *Lactobacillus* spp. to produce natural broad-spectrum antibiotics, such as lactocin, helveticin, curvacin, nisin, and ifidocin. Furthermore, FOS supplementation may cause bacteria to produce bacteriocin. Broilers fed with FOS prebiotic supplementation promoted the colonization of specific beneficial bacteria for broilers and other bacteria. *Janthinobacterium* (produce antibacterial and antifungal compounds), *Paludibacter* (propionate-producing bacteria), and *Butyrivibrio* and *Coprococcus* (butyrate-producing bacteria) are found in small amounts in the epithelial walls of ilium, leading to increased intestinal immunity and mucosal absorption area (Shang et al., 2018). This phenomenon may inhibit the growth of several pathogenic bacteria and reduce the colonization of others, such as *Salmonella* and *Campylobacter* (Sekelja et al., 2012). The increase in VFA contributed to a decrease in pathogenic microorganisms in caecum; the increase in VFA may be caused by inulin, polysaccharides, and oligosaccharides (Ahmed et al., 2018). FOS can help and maintain a healthy digestive environment by increasing the number of Bifidobacterium or decreasing the number of *E. coli* in the gastrointestinal tract (Wang et al., 2020). Several potential mechanisms for prebiotic health benefits against altering the gut microbiota have been proposed, including competitive exclusion of pathogens (Sekelja et al., 2012), antimicrobial factor production (Munoz et al., 2012), stimulation of specific immune system in animals (Babu et al., 2012), and development of intestinal morphology (Pourabedin and Zhao, 2015). Furthermore, the findings of this trial are consistent with those of previous research demonstrating the capability of synbiotics, prebiotics, and probiotics to increase the total VFA content, which improve intestinal ecology (Ahmed et al., 2018; Samanta et al., 2012). Synbiotics stimulated the growth of total and beneficial bacteria, such as LAB, Enterobacteriaceae *Bifidobacterium* and *Lactobacilli* (Al-Sultan et al., 2016; Choi et al., 2015). Furthermore, synbiotics inhibit the growth of pathogenic microorganisms, such as *Salmonella* spp., coliform bacteria, *Clostridia perfringens*, and *E. coli* (Choi et al., 2015; Çalik et al., 2017a). Moreover, synbiotic supplementation followed by probiotic supplementation enhanced microbial ecology and small intestinal morphology and more than prebiotic, and control groups (Al-Sultan et al., 2016). Nopparatmaitree et al. (2022) previously demonstrated that TABP alone could increase beneficial bacteria such as LAB, *Bifidobacterium* and *Lactobacilli* while decreasing harmful bacteria in broilers. Likewise, Nopparatmaitree et al. (2021) demonstrated an interaction effect of probiotics and TABP in laying hen diets on increasing beneficial bacteria as well as reducing harmful bacteria, such as *E. coli* and *Salmonella* spp. As a result, the findings of this experiment will be extremely useful in providing additional guidance on the use of TABP in combination with probiotics in broiler diets.

The current study also highlighted that supplementing synbiotics from TABP with 2 g/kg total probiotics in broiler diets increased the villus height, villus width, villus surface area, and cryptal depth of the jejunum compared

with the control group of broilers. Previous research has suggested that short-chain fatty acids can be used as a source of energy to promote development and the integrity of the intestinal mucosa. In this experiment, the apparent results of short-chain fatty acid content originated from the development of synbiotic-stimulated fermentation in combination with probiotics. The increase in the number of beneficial microorganisms can also reduce the number of harmful microorganisms, which directly affects villus damage because several harmful microorganisms release toxins, such as the botulinum toxin from *Clostridium botulinum* that destroy villus cells (Ahmed et al., 2018). The report described the main mechanism of prebiotics regarding immunity and the selective growth of lactic acid-producing bacteria, which result in increased concentrations of short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid, which are important as a source of energy for colon cells and stimulating the intestines (Alloui et al., 2013). Butyric acid was used as an energy source for gut microbes, acetic acid as a precursor in fat and cholesterol synthesis, and propionic acid as a precursor in the gluconeogenesis process and reduced fatty acid and lipid synthesis (Eeckhau et al., 2011). Butyric acid, on the other hand, is not only important for energizing epithelial cells but also counteracts and responds to cytokine-induced inflammation. This compound also indirectly lowers the pH of the cecum, preventing pathogen growth and increasing mineral uptake (Pourabedin and Zhao, 2015). The findings also explain the effect of synbiotics in broiler diets on the development of intestinal mucosal structure and the elevation of duodenum villus, jejunum villus (Choi et al., 2015), and ileum villus (Calik et al., 2017b), with the experimental findings similar to those of Rehman et al. (2007); Beski and Al-Sardary (2015). Moreover, increases were observed in the depth of the duodenum's crypt of Lieberkühn, jejunum (Calik et al., 2017b), ileum (Al-Sultan et al., 2016), and villus height to crypt of Lieberkühn depth ratio (Al-Sultan et al., 2016; Çalik et al., 2017a), improving the health and strength of the gastrointestinal tract (Roberfroid, 2005). The aforementioned evidence explains the scientific reasons for the use of synbiotics, which can improve intestinal ecology and promote intestinal morphological development. Increased feed utilization efficiency from TABP supplementation improved chicken growth, which resulted in a decrease in FCR over a period of 0-21 days as well as an increase in ADG over a period of 22-35 days and 0-35 days. Several previous researches have yielded similar results to this study on the use of synbiotics to increase broiler productivity, which increases the ADG of broilers in the starter (Murarolli et al., 2014; Shehata et al., 2019), finisher (Çalik et al., 2017a), and overall period (Dizaji et al., 2012; Ghasemi and Taherpor, 2013) as well as improves the FCR in the starter (Murarolli et al., 2014; Shehata et al., 2019), finisher (Çalik et al., 2017a) and overall periods (Al-Sultan et al., 2016; Dizaji et al., 2012; Ghasemi and Taherpour, 2013). The findings of this study show that synbiotics have the remarkable potential to combine the properties of probiotics and prebiotics as feed additives. This result supported previous research by Al-Sultan et al. (2016), who discovered that synbiotic supplemented groups had higher slaughter weight, body weight gain, and FCR than prebiotic supplemented groups. Tayeri et al. (2018) when compared to the control and antibiotic treatments, the probiotic, prebiotic, and synbiotic treatments increased weight gain (64, 66, 73, 70, and 74 g/d, respectively), while the synbiotic treatment decreased FCR.

This experiment used broilers raised in an open house in a tropical climate, which resulted in a higher than normal ambient temperature. The upper limit of the heating zone reduces production efficiency, reduces intestinal integrity and immune function, and results in losses in the chicken production process (Lara and Rostagno, 2013). A previous study by Hu et al. (2022) found that synbiotics supplementation reduces the negative effect of heat stress on broilers by improving productivity performance. Despite the statistical significance was not obtained, this experiment's results revealed an improvement of the survival rate and productive index. As a consequence, TABP supplementation has a potential to diminish feed costs per gain while increase net profit return and return on investment. These results of broiler performance and economic benefit return are essential indicators for the industry.

CONCLUSIONS AND RECOMMENDATIONS

Supplementation of synbiotics from TABP with probiotics in broiler diets can improve the AND of dry matter, ether extract, crude fiber, and crude protein and increase the production of short-chain VFAs in the lower gut, promoting the proliferation of LAB and *Enterococcus* sp. and decreasing the number of *Salmonella* sp. and *E. coli*. Furthermore, these synbiotics increase the villus height, villus surface area, and depth of the crypt of Lieberkühn of the duodenum, jejunum, and ileum and feed intake and broiler growth rate. This experiment demonstrated that the minimum supplementation of synbiotics from 10 g/kg TABP in combination with 2 g/kg of probiotics in diets has potential as a functional feedstuff for broilers to improve the apparent nutrient digestibility, gut ecology, and performance of broilers.

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NOVELTY STATEMENT

This research provides a novel examplifying demonstration of the synergism of synbiotics from the combination of prebiotics from TABP and beneficial microorganism as feed additives for improve apparent nutrient digestibility, gut ecology, and performance of broilers.

AUTHORS' CONTRIBUTION

MN conceptualized, collected and analyzed data, and wrote the manuscript. SB and SW data collection and husbandry work. PS data collection and laboratory work. SC editing and revising the manuscript. WK data analysis. The final manuscript was read and approved by all authors.

Conflict of interests

The authors have declared no conflict of interest.

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