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Intestinal Morphology, Protein Digestibility, and Broiler Performance Fed Encapsulated Dahlia Tuber Extract and *Bacillus subtilis*

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ABSTRACT

This study aimed to evaluate the effects of encapsulated dahlia tuber extract and *Bacillus subtilis* (EdteBs) on intestinal morphology, protein digestibility, and broiler performance. A total of 200 eight-day-old Ross 308 broilers (initial body weight: 194.60 ± 3.46 g) were divided into four dietary treatments with five replications. The treatments were: T0 – basal diet (control) without EdteBs supplementation; T1 – T0 + 0.1% EdteBs; T2 – T0 + 0.2% EdteBs; and T3 – T0 + 0.3% EdteBs. Variables measured included potential hydrogen (pH), lactic acid bacteria (LAB) population, *coliform* count, villus height, crypt depth, intestinal segment weight and height, protein digestibility, feed intake, body weight gain (BWG), feed conversion ratio (FCR), and income over feed cost (IOFC). Data were analyzed using analysis of variance, followed by Duncan's test at a 5% significance level ($p<0.05$). Results showed that EdteBs supplementation at higher levels (T2 and T3) significantly ($p<0.05$) increased LAB population, villus height, crypt depth, and intestinal segment weight and height, while pH and *coliform* counts decreased. Protein digestibility and BWG also significantly improved ($p<0.05$) in T2 and T3. Both feed intake and BWG increased, particularly in T3, contributing to lower FCR and higher IOFC. In conclusion, EdteBs supplementation enhances intestinal morphology, protein digestibility, and broiler performance, with the most effective level at 0.3%.

Keywords: ***Bacillus subtilis; broiler performance; dahlia tuber extract; encapsulation; small intestine***

INTRODUCTION

Broilers are producers of animal protein that have a short rearing period before slaughter or marketing. Broiler farming in Indonesia is growing rapidly and has increased significantly. The broiler population is projected to reach 3.12 billion birds by 2025, with a meat production volume of 3.5 million tons (Darmawan *et al*., 2023). It is also reported that the broiler population is expected to grow by an average of 1.63% per year until 2027. Therefore, broiler meat production is assumed to be capable of meeting the national meat demand. However, since broilers are fast-growing poultry that are sensitive to environmental changes, additive supplementation is needed to help prevent disease and maintain production performance.

Maintaining gastrointestinal tract (GIT) health and function is crucial, as it is closely related to productivity. The intestinal tract plays a key role in nutrient digestion and absorption (Ducatelle *et al*., 2023). One effort to improve the health and function of the broiler GIT is the use of feed additives that enhance intestinal morphology and morphometrics. A combination of probiotics and prebiotics, known as synbiotics, is a significant type of feed additive used to improve the microbial balance in the intestine and enhance poultry growth performance.

A previous study by Wang *et al*. (2021) reported that feeding *Bacillus subtilis* improved jejunal morphology by increasing villus height and crypt depth in broilers. The stimulating effects of dahlia inulin and *Lactobacillus* sp. appear to benefit not only modern poultry like broilers but also native chickens, as shown by increased villus height and growth in KUB chickens (Purbarani *et al*., 2019). Dahlia tuber extract functions as a prebiotic due to its inulin content (Mangisah *et al*., 2020; Hilman *et al*., 2021) and supports the growth of beneficial intestinal microorganisms. Krismiyanto *et al*. (2014) found that supplementing 1.17% inulin from powdered dahlia tuber increased the population of lactic acid bacteria while reducing pH and *E. coli* counts. Another prebiotic source, glucomannan extract from porang tuber, has been shown to be as effective as inulin from dahlia tuber in broilers (Perdinan *et al*., 2019) , as evidenced by improved intestinal bacterial balance, higher lactic acid bacteria counts, reduced *coliform*s, and enhanced immunity.

The synbiotic used in the present study was prepared through encapsulation using maltodextrin, a widely used and cost-effective coating material known for its beneficial properties, including high water solubility, low viscosity, and biodegradability (Šturm *et al*., 2019). Several previous studies have reported the

effects of *B. subtilis* supplementation alone (Ciurescu *et al*., 2020; Mohamed *et al*., 2022) or inulin derived from dahlia extract alone (Suthama *et al*., 2023). While a study on the combination of *B. subtilis* and inulin as a synbiotic exists, it did not involve encapsulation (Abdelqader *et al*., 2013). These previous findings highlight the novelty of the present study.

Therefore, the encapsulation of dahlia tuber extract and *B. subtilis* in this study is expected to be more effective in improving gastrointestinal tract conditions, thereby positively affecting nutrient digestibility and broiler growth. This study aimed to evaluate the feeding effect of encapsulated dahlia tuber extract and *B. subtilis* on small intestine development, protein digestibility, growth performance, and income over feed cost (IOFC) in broilers.

MATERIALS AND METHODS

The experimental protocol in this study was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (Approval No. 60-11/A-21/KEP-FPP/2024), with emphasis on supervision and compliance regarding the legal use of broilers as experimental animals. This study was also conducted following the Republic of Indonesia Law No. 41 of 2014 concerning animal health and management.

Preparation of Dahlia Tuber Extract and Encapsulation Process

Dahlia tubers were collected from plantation waste in Ungaran Regency, Semarang, Central Java. The tubers were washed and dried. The extraction procedure followed the method of Krismiyanto *et al*. (2014). The dried tubers were oven-dried at 50 °C, ground into powder, and dissolved in 70% ethanol at a 1:10 (w/v) ratio. The mixture was stirred until homogeneous, then heated in a water bath at 50 °C for 30 minutes, and filtered to obtain a white residue.

Encapsulation began with the inoculation of *B. subtilis* in a skim milk solution. Skim milk was dissolved in distilled water at a 1:2 (w/v) ratio and used as a culture medium. *B. subtilis* at a concentration of 10^{10} cfu/mL was mixed with the skim milk solution at a 1:1 (v/v) ratio, then stirred using a magnetic stirrer until homogeneous. The second step involved the preparation of a maltodextrin coating, following the method of Agusetyaningsih *et al*. (2022). Maltodextrin was dissolved in distilled water at a 1:3 (w/v) ratio. In the third step, the *B. subtilis* solution was mixed with the maltodextrin solution at a 1:5 (v/v) ratio. Dahlia tuber extract (DTE) at 1.17% of the total solution was then encapsulated. Encapsulation was carried out using a freeze-drying method at the Borobudur Extraction Center (BEC), Semarang. The freeze-drying process produced a dry clump, which was then powdered. It is assumed that *B. subtilis* viability remained stable, as the drying process did not involve heat, but freeze-drying was used instead.

Experimental Animal and Diet

This study used 200 Ross 308 broiler chickens aged 8 days, with an initial body weight of 194.60 ± 3.46 g, obtained from Charoen Pokphand Indonesia hatchery. The number of birds was determined based on the experimental design (4 treatments, 5 replications, 10 birds per replication), as outlined in the Experimental Design and Statistical Analysis section. The experiment began on day 8, considering that the gastrointestinal tract was sufficiently developed and the birds were ready for bacterial treatment. The experimental diets were formulated based on the guidelines of the National Research Council (1994), as shown in Table 1.

During the first week (days 1 to 8), the birds underwent an adaptation period and were fed a gradually adjusted combination of commercial and experimental diets. The diet ratios (%) were 75:25 on days 1–2, 50:50 on days 3–4, and 25:75 on days 5–6. On day 7, the birds received 100% experimental diet, followed by the assigned dietary treatments from day 8 to day 35. The encapsulated dahlia tuber extract and *B. subtilis* were mixed into a small portion of the feed (approximately 20 g) based on the treatment level and offered in the morning until fully consumed. Thereafter, the birds received the remaining daily feed without additives. Drinking water was provided *ad libitum*. The dahlia tuber extract contained 88.95% inulin and 91.03% carbohydrates.

Variables Measured

Potential hydrogen (pH) value and total intestinal bacteria. The pH was measured from digesta samples collected from each intestinal segment (duodenum, jejunum, and ileum) using a digital pH meter. Lactic acid bacteria and *coliform* populations in the digesta were determined by taking approximately 2 cm from each duodenum, jejunum, and ileum, followed by enumeration using the total plate count (TPC) method. Lactic acid bacteria were cultured on de Man, Rogosa, and Sharpe (MRS) medium and incubated at 37 °C for 48 hours. *Coliform*s were cultured on MacConkey agar and incubated at 37 °C for 24 hours. Both procedures followed the method of Pratama *et al*. (2021) with minor modifications.

Villus height and crypt depth. Histological analysis was conducted to measure villus height and crypt depth. Intestinal samples (duodenum, jejunum, and ileum) of approximately 3 cm length were fixed in 10% buffered formalin for 24 hours. The samples were processed using the Hematoxylin–Eosin staining method. Observations were made under a microscope connected to a digital camera at 4× magnification (Arifin & Pramono, 2014).

Length and weight of intestine. The length of each intestinal segment (duodenum, jejunum, and ileum) was measured using a measuring tape and converted to relative length units (Lan *et al*., 2020). Segment weights were measured using an analytical scale and expressed

Table 1. Composition and nutritional content of experimental diet for broiler chicken

Ingredient	Composition (%)	
	Starter	Finisher
Ground yellow corn	50.61	55.61
Rice bran	14.54	14.54
Soybean meal	24.00	19.00
Meat bone meal	10.00	10.00
Limestone	0.30	0.30
Premix	0.25	0.25
Lysine	0.10	0.10
Methionine	0.20	0.20
Total	100.00	100.00
Nutritional content		
Metabolizable energy (kcal/kg)*	3063.62	3074.29
Crude protein (%)**	21.62	19.68
Crude fiber (%)**	4.37	4.31
Ether extract (%)**	4.36	4.43
Calcium (%)**	1.03	1.09
Phosphorus (%)**	0.75	0.72
Methionine (%)***	0.48	1.07
Lysine (%)***	1.20	0.47
Arginine (%)***	1.38	1.25

Note: *Metabolisable energy was calculated using the formula by Bolton (1967) as follows: 40.81 {0.87 [crude protein + 2.25 × crude fat + nitrogen-free extract] + 2.5}. **Results of proximate analysis were obtained from the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang. ***Calculated based on the ingredient composition table.

as relative weights based on a slightly modified method of Kokoszyński *et al*. (2017).

Protein digestibility. Protein digestibility was measured using the total collection method combined with 0.5% Fe_2O_3 as a feed marker (Cholis *et al*., 2018). Excreta was fully collected for four days beginning in the fourth week of treatment (chickens aged 30 days). During collection, excreta were sprayed with 0.1 N HCl. The collected excreta was dried and ground, and 50 g of dried sample from each treatment were used for further analysis. Digestibility was calculated using the formula from Atchade *et al*. (2019):

Protein digestibility (%)=
[(protein consumption - protein excreta)/protein consumption] x 100%

Where: Protein consumption= total feed intake × % protein content of feed; Protein excreta= total excreta x % protein content in excreta.

Performance. The observed growth performance variables included BWG, feed consumption, FCR, and income over feed cost (IOFC). BWG was calculated by subtracting the initial body weight (at 8 days of age) from the final body weight and dividing the result by the number of treatment days. Feed consumption was recorded as the average daily intake per bird. FCR was determined by dividing total feed consumption by BWG.

Experimental Design and Statistical Analysis

The study was conducted using a completely randomized design with four treatments and five replications, each consisting of 10 birds. The dietary treatments were as follows: T0 – basal diet (control) without encapsulated dahlia tuber extract and *B. subtilis* (EdteBs); T1 – T0 + 0.1% EdteBs (1.2×10^7 cfu/g); T2 – T0 + 0.2% EdteBs (2.4×10^7 cfu/g); and T3 – T0 + 0.3% EdteBs (3.6×10^7 cfu/g). Data were analyzed using analysis of variance (ANOVA) at a 5% significance level. When significant differences were found, Duncan's multiple range test was used to determine differences among treatments (Steel & Torrie, 1995).

RESULTS

Potential Hydrogen (pH) Value, Lactic Acid Bacteria (LAB), and *Coliform* Populations

The dietary addition of EdteBs significantly ($p<0.05$) reduced intestinal pH in the duodenum, jejunum, and ileum of broiler chickens during both the starter and finisher periods, except in the duodenum and jejunum during the starter period, where no significant difference was observed compared to the control (Table 2). The pH values in the ileum during both periods showed similar trends, with T3 recording the lowest pH ($p<0.05$). However, in the duodenum and ileum during the finisher period, the pH values in T2 and T3 were similar and significantly ($p<0.05$) lower than those in T0 and T1.

The addition of EdteBs significantly ($p<0.05$) increased LAB populations and reduced total *coliform* counts in all intestinal segments (duodenum, jejunum, and ileum) during both periods, except in the duodenum during the starter period, where no significant difference was found (Table 2). LAB populations in the jejunum and ileum were significantly ($p<0.05$) higher in all EdteBs-supplemented groups compared to the control in both periods. The highest level of EdteBs supplementation (T3) resulted in the lowest total *coliform* count in the ileum during the starter period and in the duodenum during the finisher period.

Intestinal Segment Morphology

A significant increase ($p<0.05$) in villus height was observed in all intestinal segments during both periods in broilers fed EdteBs-supplemented diets (Table 3). According to Duncan's test, the villus heights in broilers fed 0.2% (T2) and 0.3% (T3) EdteBs were similar and significantly ($p<0.05$) higher than those in T0 and T1, except in the ileum during the finisher period, where T3 was similar to T1 (Table 3). The control group (T0) had the lowest villus height.

EdteBs supplementation also had a significant effect ($p<0.05$) on crypt depth in the intestinal segments, except in the duodenum during the starter period, where no significant difference was observed (Table 3). Supplementation at 0.2% (T2) and 0.3% (T3) levels resulted in similar and significantly ($p<0.05$) greater

Table 2. pH value, lactic acid bacteria (LAB), and *coliform* populations in intestinal segments of broiler chicken fed diets supplemented with encapsulated dahlia tuber extract and *Bacillus subtilis*

Variables	Treatments				SEM	p-value
	T0	T1	T2	T3		
Starter period						
Duodenum						
pH	5.89 [a]	5.89 [a]	5.84 [a]	5.84 [a]	0.40	0.064
BAL (log cfu/g)	7.10 [a]	7.12 [a]	7.14 [a]	7.15 [a]	0.12	0.941
Coliform (log cfu/g)	2.28 [a]	2.18 [a]	2.06 [a]	2.06 [a]	0.23	0.387
Jejunum						
pH	6.71 [a]	6.50 [a]	6.45 [a]	6.32 [a]	0.29	0.192
BAL (log cfu/g)	8.08 [b]	8.23 [a]	8.30 [a]	8.29 [a]	0.11	0.000
Coliform (log cfu/g)	3.69 [a]	3.58 [b]	3.52 [bc]	3.48 [c]	0.11	0.002
Ileum						
pH	7.04 [a]	6.85 [a]	6.69 [ab]	6.66 [c]	0.19	0.000
BAL (log cfu/g)	8.26 [b]	8.49 [a]	8.49 [a]	8.48 [a]	0.11	0.000
Coliform (log cfu/g)	3.90 [a]	3.67 [b]	3.64 [b]	3.58 [b]	0.14	0.000
Finisher period						
Duodenum						
pH	6.08 [a]	5.96 [a]	5.53 [b]	5.47 [b]	0.39	0.011
BAL (log cfu/g)	7.57 [c]	7.73 [b]	7.77 [ab]	7.87 [a]	0.13	0.000
Coliform (log cfu/g)	3.49 [a]	3.30 [ab]	3.28 [ab]	3.18 [c]	0.18	0.041
Jejunum						
pH	6.74 [a]	6.71 [a]	6.44 [b]	6.40 [b]	0.22	0.007
BAL (log cfu/g)	8.18 [b]	8.36 [a]	8.41 [a]	8.50 [a]	0.15	0.002
Coliform (log cfu/g)	3.76 [a]	3.66 [a]	3.48 [b]	3.48 [b]	0.15	0.000
Ileum						
pH	6.91 [a]	6.76 [ab]	6.75 [ab]	6.66 [c]	0.15	0.050
BAL (log cfu/g)	8.35 [b]	8.58 [a]	8.63 [a]	8.65 [a]	0.14	0.000
Coliform (log cfu/g)	3.88 [a]	3.59 [b]	3.55 [b]	3.52 [b]	0.16	0.000

Note: [abc]Mean values in the same row with different superscripts differ significantly ($p<0.05$). T0: basal diet, T1: basal diet + 0.1% EdteBs, T2: basal diet + 0.2% EdteBs, and T3: basal diet + 0.3% EdteBs.

crypt depth than T0 and T1 in both periods, except for the duodenum in the starter period.

The pattern of crypt depth followed a similar trend to that of villus height, with T2 and T3 showing the greatest values, followed by T1, particularly in the duodenum during the finisher period, the jejunum during both periods, and the ileum during the starter period. However, in the ileum during the finisher period, the crypt depth in EdteBs-supplemented groups was similar between T2 and T3 (Table 2).

The addition of EdteBs significantly ($p<0.05$) increased the intestinal length and weight during both the starter and finisher periods, except in the duodenum during the starter period, where no significant difference was observed (Table 3). In the finisher period, intestinal length and weight were significantly ($p<0.05$) increased at all levels of EdteBs supplementation (T1, T2, and T3). However, the highest values were observed only in the jejunum of T2 and T3, and in the duodenum of T3 during the starter period (Table 3).

Protein Digestibility and Broiler Performance

The addition of EdteBs to broiler diets significantly affected protein digestibility ($p<0.05$) (Table 4). According to Duncan's test, protein digestibility was significantly higher ($p<0.05$) in the T2 and T3 groups compared to T1 and the control (T0), with no significant difference between T2 and T3. However, the lowest level of EdteBs supplementation (T1) did not differ significantly from the control (T0) (Table 4).

Broiler growth performance, including BWG, feed consumption, and FCR, was significantly influenced by EdteBs supplementation (Table 4). The highest BWG was observed in the T3 group (0.3% EdteBs), although it was not statistically different from T2 (0.2%). The T2 group showed the same BWG as T1 but was significantly different ($p<0.05$) from the control (T0) (Table 4).

Feed consumption followed a similar trend to that of BWG. Both T2 and T3 groups had significantly higher feed intake ($p<0.05$) than the control (T0). However, there were no significant differences between T1 and T2, or between T0 and T1, although T3 had the highest value among all treatments (T0, T1, and T2) (Table 4). The highest level of EdteBs supplementation (T3) resulted in a significantly lower FCR ($p<0.05$) compared to the control, but it was not significantly different from T1 and T2. Similarly, FCR values for T1 and T2 were not significantly different from T0 (Table 4).

Dietary supplementation of EdteBs at 0.3% (T3) also produced the highest IOFC value ($p<0.05$), although it was not significantly different from T2. The IOFC values of T2 and T1 were similar, and both were significantly higher ($p<0.05$) than that of the control (T0) (Table 4). These results were closely related to increased feed consumption and optimal body weight gain, which contributed to lower FCR and higher IOFC.

Table 3. Villus height, crypt depth, length, and weight of intestinal segments in broiler chicken fed diets supplemented with encapsulated dahlia tuber extract and *Bacillus subtilis*

Variables	Treatments				SEM	p-value
	T0	T1	T2	T3		
Starter period						
Duodenum						
Villus height (μm)	1233.69 ^{c}	1291.23 ^{b}	1367.99 ^{a}	1387.14 ^{a}	91.81	0.015
Crypt depth (μm)	258.12 ^{a}	258.08 ^{a}	268.69 ^{a}	275.95 ^{a}	30.25	0.772
Length (cm/kg)	26.48 ^{a}	26.70 ^{a}	27.59 ^{a}	27.59 ^{a}	1.13	0.278
Weight (%)	0.81 ^{a}	0.82 ^{a}	0.85 ^{a}	0.90 ^{a}	0.12	0.608
Jejunum						
Villus height (μm)	1061.17 ^{c}	1134.62 ^{b}	1322.66 ^{a}	1335.67 ^{a}	130.42	0.000
Crypt depth (μm)	120.17 ^{b}	135.03 ^{b}	170.75 ^{a}	172.59 ^{a}	26.72	0.000
Length (cm/kg)	69.60 ^{b}	72.23 ^{ab}	74.38 ^{a}	74.94 ^{a}	2.81	0.002
Weight (%)	1.57 ^{b}	1.68 ^{ab}	1.83 ^{a}	1.87 ^{a}	0.19	0.029
Ileum						
Villus height (μm)	539.72 ^{c}	637.11 ^{b}	713.17 ^{a}	735.35 ^{a}	88.99	0.000
Crypt depth (μm)	97.98 ^{c}	106.25 ^{b}	121.67 ^{a}	128.79 ^{a}	14.03	0.000
Length (cm/kg)	74.42 ^{c}	77.45 ^{b}	77.43 ^{b}	81.88 ^{a}	3.16	0.000
Weight (%)	0.97 ^{c}	1.16 ^{b}	1.17 ^{b}	1.35 ^{a}	0.18	0.001
Finisher period						
Duodenum						
Villus height (μm)	1289.81 ^{b}	1485.01 ^{a}	1481.57 ^{a}	1565.01 ^{a}	127.20	0.000
Crypt depth (μm)	207.31 ^{c}	271.27 ^{b}	328.33 ^{a}	343.00 ^{a}	57.02	0.000
Length (cm/kg)	16.30 ^{b}	17.30 ^{a}	17.35 ^{a}	17.37 ^{a}	0.66	0.012
Weight (%)	0.59 ^{b}	0.67 ^{a}	0.69 ^{a}	0.71 ^{a}	0.06	0.013
Jejunum						
Villus height (μm)	1285.01 ^{c}	1361.38 ^{b}	1431.52 ^{a}	1448.14 ^{a}	77.50	0.000
Crypt depth (μm)	148.81 ^{c}	230.40 ^{b}	292.09 ^{a}	322.81 ^{a}	71.41	0.000
Length (cm/kg)	41.02 ^{b}	44.48 ^{a}	44.59 ^{a}	44.69 ^{a}	2.20	0.007
Weight (%)	1.15 ^{b}	1.31 ^{a}	1.32 ^{a}	1.46 ^{a}	0.15	0.007
Ileum						
Villus height (μm)	570.94 ^{c}	689.18 ^{b}	675.04 ^{b}	779.26 ^{a}	83.01	0.000
Crypt depth (μm)	176.88 ^{b}	210.37 ^{a}	211.06 ^{a}	225.35 ^{a}	24.84	0.005
Length (cm/kg)	42.30 ^{b}	46.34 ^{a}	46.92 ^{a}	47.50 ^{a}	2.83	0.005
Weight (%)	0.88 ^{b}	1.08 ^{a}	1.10 ^{a}	1.16 ^{a}	0.03	0.000

Note: ^{abc} Mean values in the same row with different superscripts differ significantly ($p<0.05$). T0: basal diet, T1: basal diet + 0.1% EdteBs, T2: basal diet + 0.2% EdteBs, and T3: basal diet + 0.3% EdteBs.

Table 4. Protein digestibility and performance of broiler chicken fed diets supplemented with encapsulated dahlia tuber extract and *Bacillus subtilis*

Variables	Treatments				SEM	p-value
	T0	T1	T2	T3		
Protein digestibility	78.68 ^{b}	80.31 ^{b}	84.45 ^{a}	85.50 ^{a}	3.22	0.000
Body weight gain (g)	56.43 ^{c}	60.10 ^{b}	62.71^{ab}	65.53 ^{a}	4.11	0.000
Feed consumption (g)	101.16 ^{c}	103.45 ^{bc}	106.80 ^{ab}	109.99 ^{a}	5.78	0.066
Feed conversion ratio	1.79 ^{a}	1.72 ^{ab}	1.70 ^{ab}	1.68 ^{b}	0.07	0.081
Income over feed cost (IDR)	17028^{c}	18461^{b}	19164^{ab}	20271^{a}	1489.52	0.001

Note: ^{abc}Mean values in the same row with different superscripts differ significantly ($p<0.05$). T0: basal diet, T1: basal diet + 0.1% EdteBs, T2: basal diet + 0.2% EdteBs, and T3: basal diet + 0.3% EdteBs.

DISCUSSION

Potential Hydrogen (pH) Value, Lactic Acid Bacteria (LAB), and *Coliform* Populations

The addition of EdteBs reduced the pH of intestinal segments (Table 2), except in the duodenum and jejunum during the starter period. The decrease in pH was attributed to the prebiotic effect of inulin extracted from dahlia tubers, which can be fermented by *B. subtilis*, supported by the activity of endogenous LAB. The acidic environment (low pH) results from metabolic products such as short-chain fatty acids (SCFA) and lactic acid generated during inulin fermentation by probiotics (Bucław, 2016). Lower pH levels are unfavorable for pathogenic bacterial growth but promote the proliferation of beneficial bacteria, particularly *Lactobacillus* spp., including lactic acid bacteria (LAB) (Table 2). These findings are consistent with those reported by Youssef *et al*. (2024), who found

that supplementation with the synbiotic PoultryStar Sol containing fructo-oligosaccharides and a probiotic bacterial blend reduced pH and decreased *E. coli* populations in Mandarah roosters.

In the jejunum during the starter period, although pH did not change significantly, LAB populations increased and total *coliform* counts significantly decreased (Table 2). This suggests that fermentation of inulin by *B. subtilis* may not have produced a large quantity of SCFA, though this was not measured, but *B. subtilis* may have exerted its probiotic effects by producing bacteriocins, which inhibit pathogenic bacteria (Liu *et al*., 2023). Bacteriocins produced by *B. subtilis* have been shown to possess antimicrobial properties that suppress the growth of pathogens in general (Ningsih *et al*., 2023), and *E. coli* and *Staphylococcus aureus* in particular (Yaderets *et al*., 2023). The inhibitory effects of *B. subtilis* on pathogenic bacteria through bacteriocin production are comparable to those of other natural additives, such as β-glucan, which functions as a prebiotic and an alternative to antibiotics for enhancing the health of animals and their products. Hashaam *et al*. (2024) recently reported that β-glucan is gaining interest as a prebiotic additive due to its positive effects on gut health by increasing *Lactobacillus* populations and improving the immune response in broilers.

The LAB population in the duodenum during the starter period did not differ significantly among treatments, possibly due to age-related factors (Table 2). The duodenum at this stage was likely still underdeveloped, resulting in a limited bacterial response to the additive despite the inclusion of inulin from dahlia tuber extract (DTE). Poultry age plays a critical role in the development of microbial populations in the digestive tract (Stamilla *et al*., 2021). There remains some debate regarding the optimal timing of additive supplementation, particularly probiotics, with some studies supporting early administration, while others suggest that intestinal bacterial development becomes more pronounced after the first week of life, as also reflected in this study. As broilers age, LAB populations in the duodenum increase while *coliform* populations decrease. Ravangard *et al*. (2017) previously reported that synbiotic supplementation enhanced *Lactobacillus* populations and suppressed *E. coli* growth in the digestive tracts of 42-day-old broilers.

The overall decrease in *coliform* populations across intestinal segments may be attributed to the protective effect of encapsulated inulin in the upper gastrointestinal tract, allowing it to reach the duodenum more effectively. *Bacillus subtilis* may also be shielded from pH fluctuations and digestive enzyme activity. As a result, the availability of inulin from DTE remains high and can be optimally fermented by *B. subtilis* in cooperation with commensal LAB, leading to SCFA production that contributes to reduced *coliform* populations, as observed in this study. Sebouai *et al*. (2024) similarly reported that when both prebiotics and probiotics reach the intestinal segment, particularly the intestine, they exert maximal effects in improving microbial balance by suppressing pathogenic bacteria.

Intestinal Segment Morphology

Dietary supplementation with EdteBs significantly increased villus height in all intestinal segments, with the most notable improvement observed at the 0.3% level (T3) (Table 3). The mechanism of probiotics or synbiotics involves not only improving gut microbial balance but also stimulating the secretion of intestinal mucus, which acts as a protective barrier and supports gastrointestinal health. Previous studies have reported that both synbiotics (Szczypka *et al*., 2021) and single-strain probiotics (Fusco *et al*., 2023) enhance the population of beneficial intestinal bacteria and inhibit pathogenic growth. These favorable physiological conditions contribute to better gut development and growth.

Additionally, butyric acid, one of the SCFAs produced through the fermentation of inulin by *B. subtilis* and commensal bacteria such as LAB, plays a crucial role in strengthening the intestinal epithelium and promoting villus growth. Leonídio *et al*. (2024) reported that dietary butyric acid positively affected villus development and intestinal integrity in broilers fed a control diet, particularly when compared to birds infected with *Salmonella enteritidis*. Although SCFA levels were not measured in the present study, the observed improvements in villus development across all segments and both periods (Table 3) support this mechanism. Previous research has shown that SCFAs produced by gut microbial fermentation of prebiotics, particularly butyrate, promote intestinal villus proliferation (Arifin & Pramono, 2014). Butyrate serves as a primary energy source for intestinal development and specifically stimulates villus growth through enhanced cellular proliferation, strengthening, and maturation (El-Saadony *et al*., 2022).

Improved bacterial balance (Table 2) likely contributed to a healthier gut environment, as reflected in enhanced intestinal development, including increased segment length and weight (Table 3). Julendra *et al*. (2020) reported that prebiotic inulin or mannan oligosaccharides (MOS), whether administered alone or in combination with *Lactobacillus plantarum*, increased villus height in broilers. Improved villus growth is closely associated with intestinal development, including length and weight. Crypt depth and villus height followed similar trends to intestinal length and weight, except in the duodenum during the starter period, where crypt depth was unaffected by EdteBs supplementation (Table 3).

Villus height is closely related to crypt depth, as taller villi are typically accompanied by deeper crypts. This phenomenon positively impacts overall intestinal growth. According to Wang *et al*. (2016), a longer intestinal tract provides a greater surface area for digestion and absorption, thereby enhancing nutrient uptake and utilization. This, in turn, supports both localized and cumulative gut development. The addition of commercial synbiotics or *Lactobacillus* spp. has been reported to increase villus height and crypt depth by accelerating the turnover cycle of intestinal epithelial cells, a process directly influenced by

probiotic activity (Awad *et al*., 2009). In this study, the enhanced epithelial cell turnover may have resulted from the activity of *B. subtilis* in EdteBs, in combination with the action of endogenous LAB. The beneficial effect of EdteBs on intestinal morphology is consistent with findings by Julendra *et al*. (2021), who reported increased villus size in broilers supplemented with a combination of *L. plantarum* and inulin at either 0.5% or 1.0%. These findings align with the present study, where improved epithelial barrier function and mucosal defense may be attributed to antibacterial compounds produced by *B. subtilis*.

Protein Digestibility and Broiler Performance

The addition of EdteBs at all levels improved protein digestibility and enhanced broiler performance, as indicated by higher BWG (Table 4). Although BWG was slightly higher in the T3 treatment group (0.3% EdteBs), it was not significantly different from the T2 group (0.2% EdteBs). The improved growth performance may be attributed to the prebiotic function of inulin, which supports microbial balance and promotes gut health. A healthy gut condition evidenced by improved villus growth (Table 3) is a key factor in intestinal function, contributing to better protein digestibility and, ultimately, increased growth. Enhanced gut conditions lead to improved nutrient digestibility, particularly of protein, which positively influences BWG (Table 4). These findings align with Afro *et al*. (2023), who reported that optimal nutrient digestibility and absorption, especially of protein, play a significant role in increasing body weight when the gut is healthy.

Increased villus height and deeper crypt depth (Table 3) offer advantages in the digestion and absorption of nutrients, particularly protein (Table 4). This is supported by Acharya *et al*. (2024), who found that synbiotic supplementation with a combination of fructooligosaccharides and four probiotic strains improved villus development, enhanced protein utilization efficiency, and increased body weight in broilers. Therefore, greater villus height (Table 3) is closely linked to the digestive and absorptive functions of the intestine due to an expanded surface area, which allows more nutrients, such as protein, to be absorbed for growth and BWG (Table 4). A previous study by Wu *et al*. (2019) also demonstrated that inulin combined with *Lactobacillus acidophilus* improved nutrient digestibility, particularly protein, and had a positive effect on broiler BWG.

In relation to protein availability, Raksasiri *et al*. (2018) reported that the combination of inulin from Jerusalem artichoke and probiotic bacteria reduced ammonia production by lowering *E. coli* populations in 42-day-old broilers. Dietary protein can be broken down into ammonia by gut pathogens such as *E. coli*, which may negatively affect the health and productivity of chickens. The present study supports this finding, as the improvement in BWG was likely associated with reduced protein degradation, as indicated by higher nitrogen retention and improved protein digestibility (Suthama *et al*., 2021). Therefore, it can be assumed that lower protein breakdown and greater dietary utilization efficiency contribute to a reduced FCR, allowing more protein to be used for BWG.

The 0.3% EdteBs treatment (T3) resulted in a lower FCR, significantly different from the control (T0) but not significantly different from T1 and T2. Conversely, the FCR value in the control group (T0), which received no synbiotic supplementation, was the highest (Table 4). This finding suggests that nutrient utilization for BWG was more efficient with EdteBs supplementation than with the control. A previous study by Afro *et al*. (2023) also demonstrated that synbiotic supplementation using soybean meal extract and *L. plantarum* reduced FCR in broilers. Similarly, the addition of EdteBs in the present study led to a lower FCR (Table 4), reflecting improved dietary efficiency in conjunction with increased BWG (Table 4).

The lowest level of EdteBs supplementation (T1) was less effective than T3 in promoting BWG. This may be attributed to the limited availability of inulin substrate at lower inclusion levels, reducing the extent of fermentation by *B. subtilis* and commensal LAB, and consequently resulting in only a mild improvement in protein digestibility and broiler growth. In contrast, the T3 treatment, with a higher EdteBs level (0.3%), provided a greater amount of inulin substrate for fermentation by *B. subtilis* and endogenous LAB, leading to a more pronounced positive effect on broiler performance. The findings of the present study, particularly those observed in the control group (T0), are consistent with Sunu *et al*. (2021), who reported that synbiotic supplementation using *Allium sativum* and *L. acidophilus* had no significant effect on BWG, feed intake, or FCR in broiler chickens when omitted.

Supplementation with encapsulated dahlia tuber extract and *B. subtilis* (EdteBs) at the 0.3% level (T3) produced the highest IOFC value 20,271 IDR, compared to 17,028 IDR in the control group (T0) (Table 4). The synbiotic improved feed utilization efficiency and BWG, resulting in a promising increase in income. When applied in large-scale commercial poultry production, this approach could offer substantial economic benefits. These results align with Wu *et al*. (2019), who reported that synbiotics are widely used as feed additives due to their beneficial effects on gut microbiota, immune function, nutrient utilization, and overall broiler performance, ultimately contributing to more economical, health-friendly meat production.

CONCLUSION

Supplementation of encapsulated dahlia tuber extract and *Bacillus subtilis* (EdteBs) at the 0.3% level (T3) improved intestinal growth and morphology, protein digestibility, broiler performance, and increased IOFC. This study suggests that further efforts may be required to produce encapsulated products in larger quantities if they are to be applied in large-scale broiler farms to achieve optimal benefits.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to any personal or financial relationships, organizations, or individuals in connection with the materials used and discussed in this manuscript.

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