Research Article

Vacuum-Dried Encapsulation of Crude Bromelain from Pineapple Peel (ECBPP) on Growth Performance, Meat Quality, and Histomorphology of Broiler Intestines

Christian Suherman[2,3], Eko Widodo[1], Irfan H Djunaidi[1], Muhammad Halim Natsir[1*]

[1]Department of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University Malang, Indonesia; [2]Doctoral Program of Animal Science, Faculty of Animal Science, Brawijaya University, Malang, Jawa Timur, Indonesia; [3]Regional Research and Development Agency of North Sulawesi Province, Indonesia.

Abstract | Pineapple peel waste is a potential source of bromelain that may improve broiler performance and feed digestibility. This study evaluated the effects vacuum-dried encapsulated crude bromelain from pineapple peel (ECBPP) as a feed additive broiler. ECBPP was produced using a vacuum-drying method with 8% maltodextrin and 2% chitosan as coating agents. A total of 308 MB 202 broilers were randomly allocated to seven dietary treatments (0%, 0.15%, 0.30%, 0.45%, 0.60%, 0.75%, and 0.90% ECBPP), each replicated four times. Parameters measured included growth performance, meat quality, and small intestinal histomorphology. ECBPP supplementation significantly improved body weight (BW) and feed conversion ratio (FCR) during the starter phase, with the 0.90% level producing the best results ($p<0.05$), showing a 4.99% increase in BW and a 2.54% reduction in FCR compared with the control. In contrast, no significant effects were observed on feed intake (FI), BW, FCR, or carcass during the finisher phase ($p>0.05$). ECBPP did not affect moisture, water-holding capacity (WHC), or L* values ($p>0.05$), but significantly increased meat color parameters at the 0.90% level ($p<0.05$), with a* values of 2.5 vs. 1.2 and b* values of 11.3 vs. 8.3 compared with the control group. Supplementation with 0.60–0.90% ECBPP improved intestinal morphology, particularly villus height, apical width, and basal width in the duodenum, jejunum and ileum ($p<0.01$). In conclusion, ECBPP supplementation, especially at 0.90%, improved growth performance during the starter phase, enhanced meat color quality, and promoted intestinal health in broilers.

Keywords | Bromelain, Growth performance, Intestinal morphology, Meat quality, Pineapple peel

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***Correspondence** | Muhammad Halim Natsir, Department of Nutrition and Animal Feed, Faculty of Animal Science, Brawijaya University Malang, Indonesia; **Email:** emhanatsir@ub.ac.id
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INTRODUCTION

The pineapple processing industry leaves solid waste such as peel, core, stem, leaves, and crown. This waste accounts for approximately 60% of the total fruit mass (Sarangi *et al*., 2023), with the peel being the largest contributor at around 30 - 40% of the total waste (Ketnawa *et al*., 2012). The management of pineapple waste poses environmental challenges but also presents opportunities for value-added utilization. Among the waste components, pineapple peel is recognized as a promising source of bromelain, an enzyme with potential applications at both community and industrial levels (Zhou *et al*., 2021).

Bromelain is a proteolytic enzyme extracted from pineapple plants (*Ananas comosus*). It has been widely used in the pharmaceutical and medical fields as both a therapeutic agent and a nutraceutical (Colletti *et al.*, 2021). As a cysteine endopeptidase, bromelain hydrolyzes peptide bonds in protein chains by utilizing sulfhydryl groups (–SH) from cysteine residues at its catalytic site, thereby supporting protein digestion into amino acids (Chakraborty *et al.*, 2021; Fissore *et al.*, 2023). In poultry, bromelain supplementation has been shown to reduce *Escherichia coli* populations in the digestive tract, improve small intestinal morphology, and enhance growth performance (Akit *et al.*, 2019; Yenice *et al.*, 2023). In ruminants, bromelain contributes to protein degradation in feed and exerts anti-inflammatory effects on intestinal tissues (Arshad *et al.*, 2014; Hale *et al.*, 2005).

The application of bromelain is limited by its stability. This enzyme exhibits autodigestion and is unstable in liquid form, particularly under acidic conditions and high temperatures, which can lead to a gradual decline in enzymatic activity over time (Ataide *et al.*, 2021; Bogsan and Todorov, 2017; Tang *et al.*, 2022). These factors pose a major challenge for the long-term use of bromelain as a feed additive. Encapsulation is a widely used strategy across various industries to protect enzymes from degradation and prolong their activity, especially under unfavorable environmental conditions (Boostani and Jafari, 2021).

The success of encapsulation is influenced by the type of coating material used. Maltodextrin is a commonly used encapsulant due to its high encapsulation efficiency and ability to maintain the stability of active compounds, including their color and antioxidant activity during storage. Its high solubility, low viscosity, low cost, and minimal acidity make it an ideal wall material (Hay *et al.*, 2025; Ribeiro *et al.*, 2020; Kosasih *et al.*, 2023). The use of a single coating material often has limitations, whereas combining two coating agents can complement each other's shortcomings. In this context, maltodextrin–chitosan is widely used as a coating material in microencapsulation because their combination improves encapsulation efficiency, storage stability, and resistance to gastric conditions, while also supporting targeted release in the intestine (Pudžiuvelytė *et al.*, 2025). The use of coating materials such as maltodextrin and chitosan in dissolution tests under simulated gastric conditions showed that the peak release of the encapsulated compound occurred between 120 and 280 minutes (Natsir *et al.*, 2019; Setiasih *et al.*, 2019). Suherman *et al.* (2025) reported that the use of a combination of maltodextrin and chitosan in crude pineapple peel extract resulted in higher yield, enzyme activity, total protein content, as well as better particle morphology and surface characteristics compared to other coating materials. Moreover, chitosan, a cationic biopolymer with bioadhesive properties, can prolong the retention time of active compounds at the target site and modulate their gradual release along the gastrointestinal tract, thereby improving absorption efficiency (Hamedi *et al.*, 2022; Maleki *et al.*, 2022).

Protein-based feed ingredients play a crucial role in livestock production as they directly affect efficiency and farmer profitability. The use of exogenous enzymes such as bromelain is a promising strategy to enhance nutrient absorption, particularly from protein-rich feed ingredients. Along with the growing awareness of food safety and antibiotic resistance, the demand for safe and sustainable feed additives has been increasing. Previous studies Akit *et al.* (2019), Yenice *et al.* (2023) and Gharib-Naseri et al. (2024) reported that the use of commercial bromelain in broilers improved performance and small intestinal histomorphology. In contrast, the present study utilized crude bromelain derived from pineapple peel waste with vacuum-drying technology at temperatures 50 °C, producing an environmentally friendly and cost-effective enzyme source. Furthermore, the profile and characteristics of crude bromelain in the form of vacuum-dried encapsulation have rarely been reported. ECBPP shows potential to enhance protein digestibility by protecting the enzyme from degradation under acidic gastrointestinal conditions, thereby improving enzyme stability in the digestive tract, supporting optimal feed utilization, and contributing to animal health and improved broiler performance.

MATERIALS AND METHODS

Animals, feed, and experimental design

The crude bromelain extract from *Ananas comosus* (L.) Merr. was obtained using the cold-press method (Suherman *et al.*, 2024) and subsequently encapsulated by vacuum drying at 50 °C under 12.35 kPa for 3 hours, with a coating mixture of maltodextrin (8%) and chitosan (2%) at a 4:1 ratio, with the coating material accounting for 30% of the total volume of crude bromelain extract, resulting in a powdered ECBPP containing 8.22% total protein, 10.67 U/mL, 112.71 U/mg specific activity, 68.62% encapsulation efficiency, an average particle size of 342 µm, and 95.33% dry matter content, as determined through laboratory analysis at the Food and Nutrition Laboratory, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia. Broilers in this study were fed a commercial basal diet obtained from PT. New Hope Group, consisting of soybean meal, fish meal, corn gluten, meat and bone meal, oil, and corn (Table 1).

This study used 308 one-day-old unsexed MB 202 Platinum broilers of the Indian River strain, with an average initial body weight of 43.93 ± 3.34 g, which had been vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) by the supplying company. The broilers were randomly assigned to seven treatment groups, each with four replications and 11 birds per replicate. All broilers were housed in the same facility and allocated into smaller pens using partitions measuring 120 × 100 × 60 cm per replication unit. The house temperature ranged between 21 and 33 °C, with the lowest temperature recorded at 21–22 °C between 2:00 and 4:00 a.m., and the highest at 30–33 °C between 11:00 a.m. and 1:00 p.m. Relative humidity was maintained between 50 and 70%, with continuous bright lighting provided inside the house and ventilation managed under a semi-open system. Day 1–7 was considered the brooding period, and supplementation with ECBPP was provided through the feed from day 8 to day 35.

Table 1: Nutritional composition of basal diet used in this study.

Feed component	Pre-Starter (days 1–7)	Starter (days 8–21)	Finisher (days 21–35)
MC (%)	13	13	13
Ash (%)	8	8	8
CP (%)	23	22	20
CL (%)	5	5	5
CF (%)	4	5	6
Ca (%)	0.8-1.1	0.8-1.1	0.8-1.1
P (%)	0.5	0.5	0.5
ME (kcal/kg)	2900	3000	3100
AA (%)			
Lys	1.30	1.20	1.05
Met	0.50	0.45	0.40
Met + Cys	0.90	0.80	0.75
Trp	0.20	0.19	0.18
Thr	0.80	0.75	0.65

Notes: Moisture Content (MC) Crude Protein (CP), Crude Lipid (CL), Crude Fiber (CF), Calcium (Ca), Phosphorus (P), Metabolizable energy (ME), Amino Acids (AA), Lysine (Lys), Methionine (Met), Methionine + Cystine (Met+Cys), Tryptophan (Trp), Threonine (Thr).

The treatment levels of ECBPP were selected based on previous studies on bromelain supplementation in poultry. Akit *et al.* (2019) reported that low doses of bromelain (0.05–0.50%) improved nutrient digestibility and gut morphology but showed limited effects on growth performance. Yenice *et al.* (2023) demonstrated that moderate inclusion levels (0.15–0.45 g/kg) significantly improved BW and FCR in broilers. In addition, Gharib-Naseri et al. (2024) showed that higher bromelain doses (0.4–0.8 mL/kg) alleviated intestinal lesions and improved feed efficiency in broilers challenged with subclinical necrotic enteritis. Accordingly, we designed a graded inclusion series from 0.15% to 0.90% ECBPP to evaluate a broad range of potential effective doses, allowing us to determine the optimal dietary inclusion rate for broiler growth, gut health, and meat quality. The treatment details are as follows:

- T0: Basal diet (BD) as a control
- T1: BD supplemented with ECBPP 0.15%
- T2: BD supplemented with ECBPP 0.30%
- T3: BD supplemented with ECBPP 0.45%
- T4: BD supplemented with ECBPP 0.60%
- T5: BD supplemented with ECBPP 0.75%
- T6: BD supplemented with ECBPP 0.90%

Growth performance

Growth performance was evaluated during the starter phase (1–21 days) and the finisher phase (22–35 days) by measuring the average Feed Intake (FI), Body Weight (BW), and Feed Conversion Ratio (FCR), with measurements taken weekly. At 35 days of age, representative broilers were randomly selected from each replicate based on the appropriate sex required for subsequent analyses and slaughtered for further evaluation.

Meat quality

A total of 28 broilers from each treatment and replicate with the same sex were slaughtered, and breast meat samples were collected and analyzed for meat quality at the Animal Product Technology Laboratory, Faculty of Animal Science, Universitas Brawijaya, Malang, Indonesia. Moisture content was determined using the gravimetric method, where empty crucibles were pre-dried at 105 °C for 1 h until a constant weight was achieved. Approximately 3–5 g of sample was placed in the crucible, dried at 105 °C for 24 h, cooled in a desiccator, and reweighed to obtain the final constant weight. Water-holding capacity (WHC) was determined using the filter paper press method. Briefly, 0.3 g of meat sample was placed on Whatman No. 42 filter paper and pressed between two glass plates with a 35-kg load for 5 min. The wet area surrounding the sample was traced on millimeter paper, and WHC was calculated as the difference between moisture content and wet area percentage. Meat color (L, a, b*) was measured on the surface of breast samples using a portable colorimeter (D65 illuminant, 10° standard observer) after 30 min blooming at 4 °C. Three readings were taken at different locations on each sample, and the average value was recorded.

Intestinal histomorphology

For intestinal histomorphology analysis, a total of 28 broilers of the same sex were slaughtered on day 35. Three-centimeter segments of the duodenum, jejunum, and ileum were excised from each replicate and fixed in 10% buffered formalin, then stored in separate containers. The samples were dehydrated through a graded ethanol series (70% to 100%), cleared in xylene (I, II, III), and infiltrated with molten paraffin. After solidification, tissue blocks were sectioned at a thickness of 5 µm using a microtome, floated on a 50 °C water bath for 15 min, and subsequently stained with hematoxylin and eosin. Histological examination was conducted at the Malang Healthy Animal Laboratory, East Java, Indonesia. The slides were examined using a Nikon Eclipse type Ei light microscope equipped with an OptiLab camera connected to a computer system. Photomicrographs were taken at 400× magnification, and measurements of villus height, apical width, basal width, villus area, and crypt depth were obtained. For each intestinal segment (duodenum, jejunum, and ileum), five different fields of view were observed, and the mean values were calculated using ImageJ software (Syamsuryadi *et al.*, 2025).

Statistical analysis

The data were analyzed using one-way analysis of variance. When significant differences were detected, Duncan's multiple range test was applied at a 5% significance level to compare the means among treatments.

RESULTS AND DISCUSSION

Growth performance

During the starter phase (1–14 days of age), FI did not differ significantly among treatments ($p>0.05$). However, BW and FCR differed significantly ($p<0.05$). At days 1–14 of age, the 0.90% ECBPP treatment (T6) resulted in the highest BW (489 g) and the lowest FCR (1.04) compared with the control and other treatments (Table 2). During the subsequent starter phase (15–21 days of age), BW and FCR differed significantly among treatments ($p<0.05$), while FI remained not significantly different ($p>0.05$). In this period, the 0.90% ECBPP treatment (T6) produced the highest BW (1074 g) and one of the lowest FCR values (1.15) compared with the control (1023 g; 1.18) and other treatments (Table 2). In the finisher phase, both at 22–28 days and 29–35 days of age, no significant differences were observed in FI, BW, or FCR ($p>0.05$) across all treatments. Nevertheless, the highest ECBPP level (T6: 0.90%) showed a trend of increased BW, particularly at 28 and 35 days of age, with a difference of up to 99 g (equivalent to a 4.26% increase) between the control group and the group receiving 0.90% ECBPP (Table 2).

Observations of carcass characteristics also showed that the addition of ECBPP did not result in significant differences ($p>0.05$). However, carcass weight increased by 65 g in the T6 group compared with the control (T0), corresponding to a 3.66% improvement (Table 4). These parameters showed numerical increases; however, the differences were not statistically significant.

In line with our study, Akit *et al.* (2019) reported similar findings, where supplementation of bromelain at a level of 0.5% in broilers resulted in significant differences in BW and FCR during the starter phase, but no significant

Table 2: Effect of dietary supplementation with ECBPP on Feed Intake (FI), Body Weight (BW), and Feed Conversion Ratio (FCR) of broilers during the starter phase (Days 1–14 and 15–21).

Days 1–14	**FI (g)**	**BW (g)**	**FCR**
T0	510±1.75	463±13.68[a]	1.10±0.03[a]
T1	510±1.52	468±7.33[a]	1.09±0.02[ab]
T2	511±1.83	471±8.82[a]	1.09±0.02[ab]
T3	510±1.70	480±10.97[ab]	1.06±0.02[bc]
T4	508±3.24	463±6.91[a]	1.10±0,02[ab]
T5	509±1.87	470±10.68[a]	1.09±0.02 [ab]
T6	510±1.94	489±10.81[b]	1.04±0.02[c]
p-Value	0.625	0.023	0.031
Days 15–21	FI (g)	BW (g)	FCR
T0	1212±19.84	1023±15.29[a]	1.18±0.01[ab]
T1	1229±5.51	1020±13.95[a]	1.21±0.02[b]
T2	1208±28.18	1019±5.61[a]	1.19±0.03[ab]
T3	1212±9.06	1044±21.44[ab]	1.16±0.02[b]
T4	1188±14.59	1029±12.76[a]	1.15±0.02[b]
T5	1219±35.58	1038±29.78[a]	1.17±0.01[ab]
T6	1239±27.21	1074±21.28[b]	1.15±0.01[b]
p-Value	0.202	0.016	0.029

Notes: Data are presented as means ± SD. Different superscript letters (a–c) within the column indicate significant differences.

Table 3: Effect of dietary supplementation with ECBPP on Feed Intake (FI), Body Weight (BW), and Feed Conversion Ratio (FCR) of broilers during the finisher phase (Days 22–28 and 29–35).

	FI (g)	**BW (g)**	**FCR**
Days 22–28			
T0	2186±38.76	1560±35.94	1.40±0.03
T1	2219±15.52	1634±57.92	1.36±0.04
T2	2202±100.1	1619±43.11	1.36±0.06
T3	2152±10.34	1607±16.59	1.34±0.02
T4	2161±34.72	1624±31.21	1.33±0.03
T5	2219±71.03	1619±103.6	1.37±0.06
T6	2261±76.15	1683±33.01	1.34±0.05
p-Value	0.321	0.244	0.549
Days 29–35			
T0	3309±63.50	2326±28.76	1.42±0.02
T1	3361±29.68	2375±85.04	1.41±0.06
T2	3297±93.79	2385±52.82	1.39±0.06
T3	3246±18.07	2377±41.42	1.37±0.02
T4	3303±56.14	2376±27.45	1.39±0.04
T5	3334±131.7	2391±70.42	1.39±0.05
T6	3419±76.61	2425±45.14	1.41±0.05
p-Value	0.201	0.525	0.614

Notes: Data are presented as means ± SD.

Table 4: Carcass characteristics of broilers fed with crude bromelain encapsulated feed additive.

Treatment	Carcass weight (g)	Carcass (%)
T0	1778±38.87	76±1.81
T1	1728±154.1	73±5.36
T2	1745±44.30	73±1.43
T3	1809±58.88	76±1.74
T4	1831±75.42	77±2.38
T5	1844±112.4	77±7.27
T6	1843±79.81	76±1.09
p-value	0.54	0.62

Notes: Data are presented as means ± SD.

differences in FI, BW, or FCR during the finisher phase. Similarly, Gharib-Naseri et al. (2024) reported that supplementation with 0.8 g of bromelain had a positive effect on broilers aged 11–14 days, with significant improvements in FCR and BW, but no significant effects on FCR or BW in broilers aged 15–35 days. Furthermore, Aderibigbe *et al*. (2020) reported that protease with an activity of 9,000 TIU/g was able to improve BW in broilers at 7, 14, and 21 days of age. However, our findings demonstrated minor differences when compared with other studies, Yenice *et al*. (2023) reported that bromelain supplementation up to 0.45 g/kg improved FI and FCR from weeks 3 to 6, while BW was only significantly increased at week 6.

Several studies have shown that broilers in the starter phase have not yet developed optimal endogenous enzyme activity, as indicated by lower activity of endogenous enzymes such as proteases and reduced nutrient digestibility compared to broilers in the finisher phase (Alqhtani *et al*., 2022). Other studies have also reported that supplementation with exogenous enzymes helps improve protein digestion, nutrient absorption, and growth performance, particularly during the starter phase (Giacobbo *et al*., 2021). These findings are consistent with our study, which demonstrated that bromelain significantly affected body weight gain and improved FCR in broilers during the starter phase. In contrast, during the finisher phase, broilers possess optimal endogenous enzyme activity, as reported by Akit *et al*. (2019), which may explain why ECBPP supplementation did not result in significant differences in growth performance or carcass characteristics.

The duration of the rearing period and the use of feed additives can influence the growth performance of broilers. Yenice *et al*. (2023) reported that bromelain supplementation did not significantly affect body weight or weight gain of broilers during weeks 4 and 5, but a significant improvement was observed in week 6. In addition, the total protein content and enzymatic activity of the supplementation may affect catalytic efficiency, where higher enzyme purity and activity contribute to greater effectiveness in the digestive tract (Robinson, 2015).

Meat quality

Analysis of the physical and chemical quality of breast meat showed that moisture content, WHC, and lightness (L*) did not differ significantly among treatments ($p>0.05$). However, meat color parameters a* and b* differed significantly ($p<0.05$), particularly in the 0.90% treatment group (Table 5).

Consistent with our findings, Yenice *et al*. (2023) and Akit *et al*. (2019) reported that bromelain supplementation significantly affected meat color parameters, including L*, a*, and b*, in both breast and thigh muscles of broilers. The increase in a* and b* values observed in our study may be associated with bromelain-induced degradation of muscle structure and the breakdown of myoglobin, which is subsequently converted into oxymyoglobin or metmyoglobin. This process contributes to the enhancement of red (a*) and yellow (b*) color intensities in meat tissue (Santos *et al*., 2020).

In addition, pineapple peel of the Queen variety is known to contain carotenoids, particularly (all-E)-lutein (a xanthophyll) and (all-E)-β-carotene, with relatively

Table 5: Physical and chemical quality of broiler breast meat fed with crude bromelain encapsulated feed additive.

Treatment	Moisture	WHC	L*	a*	b*
T0	76.40±1.11	32.93±1.59	53.01±2.78	1.28±0.30^{a}	8.38±0.84^{a}
T1	75.78±0.58	31.86±4.39	54.18±1.44	1.30±0.47^{a}	9.55±0.67^{ab}
T2	76.47±1.34	32.98±3.44	52.80±2.36	1.18±0.31^{a}	9.77±1.41^{ab}
T3	75.66±0.98	31.30±2.17	50.51±2.62	2.34±0.69^{bc}	8.62±0.94^{a}
T4	75.55±0.44	25.47±3.44	52.56±3.62	1.49±0.35^{ab}	8.95±0.84^{a}
T5	74.67±0.98	27.23±5.02	54.13±0.83	1.53±0.51^{ab}	9.81±0.78^{ab}
T6	75.77±0.62	32.29±2.94	53.53±2.17	2.58±0.94^{c}	11.32±1.24^{b}
p-value	0.30	0.07	0.57	0.02	0.03

Note: Data are presented as means ± SD. Different superscript letters (a–c) within the same column indicate significant differences. WHC: water-holding capacity; L*: lightness; a*: redness; b*: yellowness.

Table 6: Histomorphology of the broiler small intestine fed with crude bromelain encapsulated feed additive.

Treatment	Villi height (µm)	Apical width (µm)	Basal width (µm)	Villi Area (µm)	Crypt depth (µm)	VH:CD (µm)
Duodenum						
T0	733±93.68[a]	71±13.80[abc]	83±13.46[ab]	1761±157	90±7.19	8.6±1.67
T1	804±80.19[ab]	60±11.93[a]	79±7.64[a]	1965±257	91±8.68	9.3±1.06
T2	768±81.51[ab]	64±11.19[ab]	92±4.43[abc]	1924±172	99±9.45	9±1.27
T3	860±40.13[ab]	82±6.86[bcd]	89±9.79[abc]	2234±85	102±9.12	10.8±1.79
T4	885±85.21[b]	98±11.85[d]	108±8.43[c]	2190±203	106±3.20	10.2±2.55
T5	1021±40.03[c]	84±11.87[cd]	110±16.25[c]	2239±251	106±6.74	9.4±1.86
T6	877±40.92[b]	89±6.90[cd]	104±7.17[bc]	2156±243	107±11.7	8.5±1.37
p-value	0.00	0.00	0.03	0.50	0.09	0.30
Jejunum						
T0	767±89.56	67±14.74[a]	105±28.88	1821±219	108±16.44	5.9±0.49
T1	753±96.48	63±17.05[a]	125±17.01	1855±219	118±11.66	5.6±1.36
T2	768±107.2	81±8.28[ab]	125±52.06	1950±246	113±7.98	6.3±0.78
T3	783±111.1	93±13.87[bc]	125±34.17	1867±190	116±14.82	6.2±1.60
T4	921±103.6	106±11.43[c]	153±26.95	2297±175	128±12.95	7.2±1.77
T5	881±56.56	112±13.95[c]	164±14.55	2016±248	125±27.17	6.8±11.1
T6	776±88.92	113±10.21[c]	152±38.63	2022±276	133±21.95	6.6±0.41
p-value	0.24	0.00	0.31	0.73	0.56	0.74
Ileum						
T0	541±67.83[a]	51±3.67[a]	105±22.29	1740±113	87±14.79	6.9±1.33
T1	524±86.81[a]	51±8.77[a]	114±13.71	1797±259	88±9.59	6.8±1.04
T2	553±54.11[a]	63±17.9[ab]	101±10.34	1612±266	85±6.94	6.9±1.58
T3	725±93.17[b]	73±5.51[abc]	115±37.71	1653±206	88±12.63	6.9±1.20
T4	945±28.19[c]	91±29.2[bc]	140±38.06	1963±215	111±14.62	7.8±1.57
T5	784±60.28[b]	80±24.9[abc]	125±24.81	1695±243	91±6.42	8.9±0,48
T6	838±98.97[bc]	100±13.31[c]	173±35.01	2150±256	107±28.56	7.8±1,24
p-value	0.00	0.01	0.06	0.37	0.61	0.37

Note: Data are presented as means ± SD. Different superscript letters (a–d) within the same column indicate significant differences. VH:CD (Villus Height: Crypta Depth).

high concentrations of approximately 565 µg/100 g fresh weight (Steingass *et al*., 2020). The presence of these xanthophylls is important because such pigments play a role in determining the intensity of broiler meat color. In general, broiler meat color is strongly influenced by dietary pigments, especially xanthophylls, which are the primary contributors to yellow coloration. The absorption of xanthophylls is highly dependent on intestinal integrity, where increased villus height and larger absorptive surfaces facilitate more efficient uptake of these pigments. Improved gut integrity has been shown to correlate positively with elevated concentrations of carotenoids and xanthophylls in plasma, which are subsequently deposited in muscle tissue and contribute to higher a (redness) and b (yellowness) values in broiler meat (Cho *et al*., 2024). Thus, the presence of carotenoids from pineapple peel, acting as dietary pigments, together with the proteolytic activity of bromelain in improving intestinal morphology, may synergistically enhance pigment deposition and color stability, thereby potentially leading to improved meat color quality in broilers.

Intestinal histomorphology

Histomorphological analysis of the duodenum showed no significant differences ($p>0.05$) in villus area, crypt depth, and the villus height to crypt depth (VH:CD) ratio. However, significant differences ($p<0.01$) were observed in villus height, apical width, and basal width in treatments T4, T5, and T6. In the jejunum, no significant differences ($p>0.05$) were found in villus height, basal width, villus area, crypt depth, or VH:CD ratio. Nevertheless, apical width was significantly affected ($p<0.01$) in treatments T4, T5, and T6. Similarly, in the ileum, there were no significant differences ($p>0.05$) in basal width, villus area, crypt depth, or VH:CD ratio. However, villus height and apical width showed highly significant differences ($p<0.01$) in treatments T4, T5, and T6 (Table 6 and Figure 1).

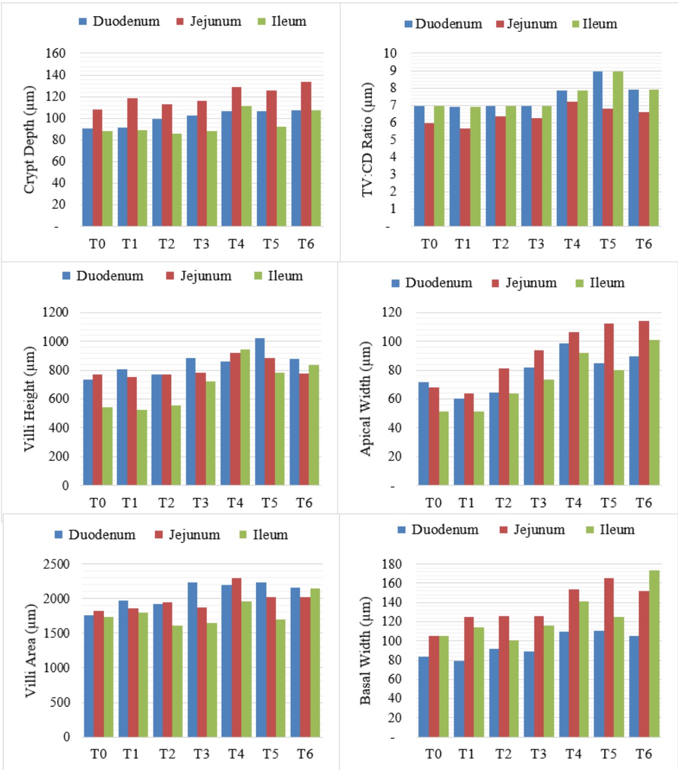

Figure 1: Comparative graph of the duodenum, jejunum, and ileum histomorphology for all treatments.

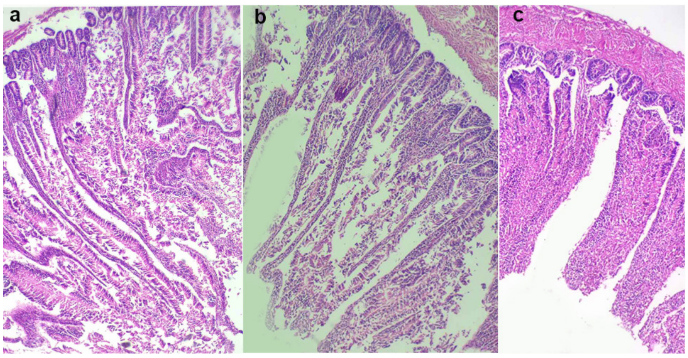

Figure 2: Histopathological images of the small intestine of broilers in the T6 group fed with ECBPP supplementation: (a) duodenum, (b) jejunum, and (c) ileum, observed at 400× magnification.

This study demonstrated significant differences in intestinal histomorphology parameters, particularly in villus height, apical width, and basal width in the duodenum and ileum, as well as apical width in the jejunum (Figure 2). The results indicate that higher levels of ECBPP in broilers are associated with greater improvements in villus height and width, especially in the duodenum and ileum, suggesting enhanced nutrient absorption capacity and improved gut health. These findings are consistent with previous research by Akit *et al.* (2019), who reported that bromelain supplementation reduced *Escherichia coli* populations, increased *Lactobacillus* spp. counts, and improved villus height in broilers. In addition, Yenice *et al.* (2023), noted that bromelain's therapeutic effects contribute to the integrity of the intestinal mucosa by inhibiting the growth of pathogenic bacteria and protecting mucosal tissue from damage. An essential element in nutrient utilization from feed is the surface area of the intestinal villi, where nutrients are transported into the bloodstream and determine the animal's capacity for absorption. Taller and wider villi provide a greater absorptive capacity, thereby facilitating more efficient nutrient transport across their surfaces (Novotný *et al.*, 2023).

Enzymes can influence intestinal morphology by reducing digesta viscosity, thereby stimulating the growth and renewal of epithelial cells. This condition supports the development of longer villi and improved crypt structure, ultimately enhancing the absorptive capacity of the intestine. In addition, enzymes are also known to increase the production of short-chain fatty acids, and several studies have demonstrated that protease supplementation exerts positive effects on gut morphology (Soriano *et al.*, 2025). Beyond its proteolytic action, bromelain also exhibits anti-inflammatory and antioxidant activities that further contribute to the improvement of intestinal structure. Lee *et al.* (2018) reported that bromelain can inhibit the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and modulate the MAPK signaling pathway, which is involved in the inflammatory response. In addition, the antioxidant activity of bromelain is demonstrated by its ability to neutralize free radicals such as DPPH and H_2O_2, thereby reducing oxidative stress and protecting mucosal cells from damage.

CONCLUSION

ECBPP supplementation influenced the growth performance of broilers during the starter phase, with the 0.90% level producing the highest BW and the lowest FCR, whereas no significant effects were observed during the finisher phase. In addition, this level had a positive effect on meat quality by increasing the a* and b* color values. Furthermore, 0.90% ECBPP supplementation also improved intestinal histomorphology, particularly by increasing villus height, apical width, and basal width in the duodenum and ileum, as well as apical width in the jejunum.

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NOVELTY STATEMENT

This study highlights the novelty of utilizing pineapple peel from the Queen variety in Kediri, East Java, Indonesia as a source of bromelain. The research uniquely applies a low-temperature vacuum drying method (50°C) for enzyme encapsulation using maltodextrin–chitosan, and directly evaluates its application as a functional feed additive in broilers.

AUTHOR'S CONTRIBUTION

All authors contributed to the conceptualization, methodology, resources, and formal analysis; Christian Suherman performed data curation, investigation, visualization, and writing original draft; Eko Widodo contributed to investigation, supervision, and review and editing; Irfan H. Djunaidi contributed to supervision and review and editing; and Muhammad Halim Natsir contributed to project administration, supervision, and review and editing.

Ethical approval

This study has passed the Ethical Clearance stage from the Animal Care and Use Committee of Brawijaya University Indonesia with number 134-KEP-UB-2024 dated July 29, 2024.

Generative AI and AI-assisted technology statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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