(Original Research)

The Effects of *Piper Betle* and *Alpinia galanga* Feed Additives on Growth Performance, Carcass Characteristics and Histomorphometry of Small Intestine in Broilers

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Abstracts

Background: The potential in combining *Piper betle* (betel) and *Alpinia galanga* (galangal) into a polyherbal formulation in broiler feed is currently unknown despite having numerous individually-established positive effects. The study aims to investigate the effects of the betel and galangal on growth performance, carcass characteristics, and histomorphometry of the small intestine. Methodology: Sixty day-old broiler chicks were randomly grouped into R1 (0.50% betel, 1.50% galangal), R2 (1.00% betel, 1.00% galangal), R3 (1.50% betel, 0.50% galangal) and baseline control group (BC). Weekly growth performance was monitored using FCR. On day 35, carcass characteristics were evaluated after slaughter. Two to three cm of duodenum, jejunum, and ileum were collected for histomorphometry. Statistical one-way ANOVA, Tukey, Kruskal-Wallis and Dunn tests were used. Results: The growth performance showed no significant changes. For carcass characteristics, the dressing percentage of the R1 group significantly increased ($p<0.001$) at 3.58%, while the R2 group significantly increased ($p<0.001$) at 3.09%. Additives in R1, R2, and R3 significantly improved ($p<0.001$) the histomorphometry of duodenum and jejunum, which include villi height, villi width, crypt depth, villi surface area and tunica muscularis thickness. Conclusion: In conclusion, the addition of betel and galangal powder at 2% of broiler feed improves both carcass characteristics and small intestine histomorphometry.

Keywords

Piper betle, Alpinia galanga, histomorphometry, carcass characteristics, phytogenic feed additive

1. Introduction

Phytogenic feed additives (PFA), phytobiotics or botanicals are described as various plant secondary compounds and metabolites that benefit animal health and production, including feed and animal products [1]. Many types or forms of medicinal plants, herbs, and spices are prepared to

be incorporated as feed additives. One form of PFA administered to the animals is the dried and grounded form or powder [2] [3].

Polyherbal formulations are described as formulations of two or more herbs that are considered more advantageous hypothetically over conventional single-component formulations due to the inadequacy of the active phytochemical constituent from individual plants to achieve desirable effects [4] [5] [6]. This theory is supported by the evidence that crude plant extracts are typically more potent than isolated constituents [5]. Even traditionally, whole plants or mixtures are used as medicine, not in isolated compound form. Another benefit of using polyherbal formulation is that besides enhanced therapeutic effects, the combination of multiple herbs in a lower and meticulous ratio will reduce the risk of harmful side effects and toxicity [4] [5] [6]. The polyherbal formulation provides benefits due to synergistic effects in terms of pharmacodynamic or pharmacokinetic mechanisms. The synergistic pharmacokinetic mechanism occurs when the plant eases the absorption, distribution, metabolism and elimination of the other plants [5]. In comparison, synergistic pharmacodynamic mechanism refers to the enhanced or amplified overall effect that is more remarkable than the sum of the pharmacological effects of each agent in the combination [7]. Polyherbal formulation also allows single administration of various plants, which is more convenient, leading to better compliance and therapeutic effects [5].

Currently, numerous studies have investigated the effects of polyherbal formulations in poultry. Out of 31 studies listed in a systematic review on phytobiotics in broiler nutrition, 22 focused on polyherbal formulations from various plant species, spanning from 2014 to 2019 [8]. The review reported that the majority of phytobiotic studies used *Origanum vulgare* (30.3%), *Thymus vulgaris* (24.24%), *Allium sativum* (15.15%), *Pimpinella anisum* (15.15%), *Mentha arvensis* (12.12%), *Cinnamomum* sp. (9.09%) and *Citrus limonum* (9.09%) [8]. Positive effects of polyherbal formulations on broilers were established in numerous publications. For instance, polyherbal mixtures of traditional Chinese medicine, including *Portulaca oleracea* L., Radix *Sophora flavescens*, *Thalictrum glandulosissimum*, *Terra flava usta*, and *Pogostemon cablin* were shown to improve growth performance, antioxidant capacities, immune function, intestinal morphometry, and intestinal barrier function in yellow-feathered broilers [9]. In another study, the supplementation of commercial polyherbal formulation Kolin Plus(tm) that comprised of *Acacia nilotica* and Curcuma longa plant parts had exerted hepatoprotective effects on broilers induced with fatty liver syndrome [10].

As one of the economically and medicinally important plants, *Piper betle* (betel) has been cultivated in Southeast Asia for religious, cultural and medicinal purposes. The main part of the typically used plants is the glossy heart-shaped leaves. Phytoconstituents of this plant include compounds from alkaloids, glycosides, tannins, phenols, flavonoids, terpenes and oligosaccharides [11]. Likewise, the use of *Alpinia galanga* (galangal) worldwide in treating diseases and food flavorings is very popular, as they are widely grown in Asia. The most used and researched part is the galangal rhizome, although the flower part also has some beneficial compounds [12]. Mainly, the phytochemicals of galangal rhizomes consist of terpenes and phenols [12].

Previously, both betel and galangal had recorded significant positive impacts as PFAs in broilers mainly on growth performance, intestinal morphometrics and blood biochemistry, but not being administered or studied as pair [13] [14] [15] [16] [17] [18] [19]. No research has been conducted on polyherbal formulations exclusively combining these two herbal plants as feed additives in broilers [20]. There is a need to study the effects of polyherbal formulation in feed to broilers because of the significant changes to the phytochemical constituents from different plants when combined as compared to separate administrations. Previous studies have reported different inclusion percentages of betel and galangal respectively in broiler feed for best performance. The optimal ratio after combining both plants as feed additive is currently unknown and has not been studied. The potential of administering these plants as feed additives to improve broiler performance is still questionable and not convincing due to inadequate

scientific evidence to support the practice. In response, the study aims to assess the effects of the betel and galangal polyherbal formulations on the growth performance, carcass characteristics and histomorphometry of the small intestine in broilers to be utilized as phytogenic feed additives.

2. Materials and Methods

2.1 Preparation of Betel Powder and Galangal Powder

Eight kilograms of fresh betel leaves were purchased from a Malaysian Good Agricultural Practices (myGAP) certified betel farm in Kuala Pilah, Negeri Sembilan. The betel leaves were air-dried and sun-dried to remove excessive water residues from the surface before being dried inside the incubator at the optimum temperature of 70 °C, for at least 1 hour [21]. Subsequently, the dried brittle leaves were crumbled into smaller pieces and ground into powder form using a blender (Le Chef Smart Essence Blender, SSL-766). The yield of ground betel was about 2 kg of moderately coarse textured powder, which was stored in a dry area at room temperature and kept away from direct sunlight.

Three kilograms of fresh greater galangal rhizomes were bought at a local wet market in Kota Bharu, Kelantan. The rhizomes were washed with water, peeled, cut, sliced into small pieces, and air-dried before freeze drying. Next, the freeze-dried galangal little pieces were ground into powder form using a blender. Approximately 2 kg of moderately coarse textured galangal powder was then stored in a manner similar to the betel powder.

2.2 Animal Management

The animal experiment was conducted following the Ethical Committee for the Experimental Use of Animals at the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan (UMK), (UMK/FPV/ACUE/PG/002/2022). Sixty day-old Ross broiler chicks were purchased from a commercial hatchery. The broiler chicks were individually weighed (average weight at day-old age was 58.3 g) and divided into four groups using a completely randomized design experimental model. The stocking density of 15 birds per cage was used to rear the broilers in raised metal cages at 1.83 m length × 0.76 m width × 0.48 m height in the Animal Research Laboratory, Faculty of Veterinary Medicine, UMK. The room temperature was initially set at 30 ± 1 °C on day 1, then gradually decreased to approximately 25 ± 1 °C by day 14. The average relative humidity ranged from 61 to 90% throughout the experiment. The broilers received continuous fluorescent lighting from 6:00 in the afternoon to 8:00 in the morning. To ensure adequate and equal feed consumption, the daily amount of feed given was based on the as-hatched performance for Ross 308 broilers by Aviagen [22]. The day-old chicks were fed a starter diet from day-1 to day-21 and a grower diet from day-22 to day-35. The composition of the starter and grower feed listed by the manufacturer was corn, soybean meal, other grains and grain by-products, animal protein, vegetable oil, salt, calcium carbonate, coccidiostats and approved antimicrobials. The starter and grower feeds for three groups were supplemented with 2% of betel and galangal powders and labelled as R1 (0.50% betel, 1.50% galangal), R2 (1.00% betel, 1.00% galangal) and R3 (1.50% betel, 0.50% galangal). Only one group was not supplemented and fed with basal starter and grower diet, the baseline control group (BC). The nutritional compositions of broiler feeds at the starter phase and grower phase added with different levels of betel and galangal powders combination are shown in Supplementary Table 1 and Supplementary Table 2 respectively. On day-35, all broiler chickens were slaughtered according to the Halal slaughter method set by the Malaysian standards on Halal food requirements (MS 1500: 2019).

2.3 Assessment of Growth Performance

The body weights of broilers (BW) were recorded weekly and compared to the initial body weights to assess the weekly body weight gain (BWG). The feed intake (FI) was also obtained weekly by recording the consumed amount by each group. Based on the weekly BWG and FI data, the feed conversion ratio (FCR) was calculated to determine the growth performance for all groups. Weekly weighing sessions were fixed on day-1,

day-7, day-14, day-21, day-28 and day-35. The formula of BWG, FI and FCR were calculated as follows:

BWG (g) = Weekly BW (g) - Initial BW (g)

FI (g) = Weight of feed Supplied (g) - Weight of left-over feed (g)

FCR = FI (g) / BWG (g)

2.4 Assessment of Carcass Quality

On day 35, various parameters were measured, including final live weight, kill-out weight, de-feathered weight, dressing percentage and the weights of selected parts that include breast, drumstick, wing, head, neck, shank, heart, liver, proventriculus, full gizzard, empty gizzard and total gastrointestinal tract (GIT) weight [23] [24][25]. The dressing percentage was determined by calculating the percentage of the dressed poultry carcass weight (after the removal of the head, shank, and all internal organs except the kidneys) from the final live weight. The cutting of different parts was standardized according to the commercial standard cuts. The shank was removed by cutting at the tarsal joint of carcass. After the head was removed at the atlas joint, the neck part was obtained by cutting near the last cervical vertebra. To weigh the breast, the breast portion (including the keel bone) was cut across at the middle through the soft part of the breast bone parallel to the seventh rib. Next, the drumstick cut (distal portion of the whole leg) was obtained by cutting through the hock joint separating the thigh and the drumstick. For the wing cut, the carcass was cut at the base of the humerus that included the whole wing and a portion of the shoulder meat.

2.5 Histomorphometry of Villi and Tunica Muscularis in Small Intestine

After measuring the weight and length of the intestinal segments, 2 to 3 cm of the duodenum, jejunum and ileum from every broiler were collected for histomorphometric analysis. Based on the intestinal segment demarcations described by [14] one representative from the cranial, middle and caudal duodenal segments was harvested from each duodenum sample that was identified starting from the pylorus and ending at the distal part of the duodenal loop. Similarly, one representative from the cranial, middle and caudal jejunal segments was harvested from the jejunum that was identified starting from the distal portion of the duodenal loop and ending at the Meckel's diverticulum. One representative from the cranial, middle and caudal ileal segments was harvested from the ileum that was identified starting from Meckel's diverticulum and ending at the anterior part of the ileocecal junction. Each of the cranial, middle and caudal segments of the duodenum, jejunum and ileum were flushed with 0.9% NaCl or normal saline and preserved in 10% neutral buffered formalin solution for 48 hours. The tissues were then processed using routine histological technique and stained with Hematoxylin and Eosin. The morphometric parameters were observed using a microscope with an image analyzer (Olympus BX51TF-CCO-FL, Japan) to capture the micrographs for analysis using the Olympus cellSens Standard Imaging Software.

For every broiler, parameters from nine intestinal tissue samples were analyzed which were the cranial, middle and caudal segments of duodenum, jejunum and ileum. The villi and crypt parameters for each intestinal tissue sample were determined by calculating the average of five different intact full finger-shaped villi and crypts selected randomly. Measurement of villi height was determined from the base of villi-crypt transition to the tip [26], whereas villi width was measured at half villi height [26] (Appendix Fig. 1). As for the crypt depth, the invagination between two villi from the base of the villi-crypt transition to the base of the crypt was measured [27] [28] (Appendix Fig. 1). Determination of villi length to crypt depth ratio was derived by dividing the villus height by crypt depth [26]. Villi surface area was calculated according to the formula, $2p \times (\text{villi width}/2) \times \text{villi height}$ [27]. For the tunica muscularis thickness, five measurements were recorded from the distance between the lamina muscularis mucosae internally and the tunica serosa externally per intestinal segment [29] (Appendix Fig. 2).

2.6 Histomorphometry of Goblet Cells in Small Intestine

The cranial, middle and caudal segments of duodenal, jejunal and ileal tissue samples were also stained with Alcian blue and PAS stains to differentiate between neutral and acidic mucosubstances according to the standard protocol in Pathology Research Lab, Universiti Sains Malaysia, USM (MS ISO 15189). The preparation of the tissue histological slides was similar to routine procedures, except for the staining and subsequent procedures. The slides with the mounted sections were heated on the hot plate to remove excess wax. Then, the slides were immersed in xylene for deparaffinization and 100%, 95%, 80% and 70% ethanol for rehydration. After the slides were rinsed with distilled water, the slides were put in the Alcian blue solution (pH 2.5, 3% aqueous acetic acid) for 30 minutes at 4 °C. Next, the slides were rinsed with distilled water to remove excess stain before being immersed in the 1% periodic acid for 10 minutes. After rinsing in distilled water, the Schiff's solution was put on the slides and left for 20 minutes. Subsequently, the slides were rinsed with distilled water before being immersed in Harris Hematoxylin for one minute. Finally, the slides were rinsed with distilled water, then dehydrated in 100% ethanol, cleared with xylene and mounted with mounting media.

The mounted slides stained with the combined Alcian-Blue and PAS stain were used to count the goblet cells that were recognized as narrow-based cells with wide, apical portions using a microscope with an image analyzer (Olympus BX51TF-CCO-FL, Japan). Five different villi per cross-section were chosen to count the total goblet cells, blue stained goblet cells containing acidic mucin, magenta-stained goblet cells containing neutral mucin and purple-stained goblet cells containing mixed acidic and neutral mucin as shown in Appendix Fig. 3 [30]. Goblet cells were counted using the object counting feature in the Olympus cell Sens Standard Imaging Software.

2.7 Data Analysis

The data analyses were done using the IBM SPSS Statistics 26 software (Statistical Package for the Social Science). The results were presented as mean ± standard error (SE). The normally distributed data in this study was analyzed by parametric one- way ANOVA test. All differences among means were considered significant at $p<0.05$ using the Tukey post hoc test. The data that was not normally distributed was analyzed using the non-parametric Kruskal-Wallis test and pairwise comparisons of Dunn's post hoc test with Bonferroni adjustments, which were considered significantly different at $p<0.05$. The analysis of the association between the two variables was determined by using Pearson's or Spearman's correlation coefficient which was considered significantly different at $p<0.05$.

3. Results

3.1 Assessment of Growth Performance

The assessment of growth performance for all the broilers is illustrated in Table 1. There was no statistically significant difference ($p>0.05$) in all the parameters evaluated.

3.2 Assessment of Carcass Quality

The assessment of carcass quality of all the broilers is recorded in Table 2. Overall, the dressing percentages of broilers included with R1 and R2 additives were significantly higher ($p<0.05$) than control and R3. The mean dressing percentage of R1 group (75.12%) significantly increased ($p<0.001$) at 3.58%, while the R2 group (74.63%) significantly increased ($p<0.001$) at 3.09% from the baseline dressing percentage (71.54%) in this study. For the edible cuts, there were significant variations between experimental groups in the edible cuts. The R3 group had significantly heavier ($p<0.05$) drumstick weight than the R1 drumsticks. Meanwhile, R2 wings were significantly heavier ($p<0.05$) in weight than R3.

Regarding edible giblets (liver, heart, gizzard), R1 carcasses had significantly heavier ($p<0.001$) liver and heart, whereas R2 carcasses had significantly heavier ($p<0.001$) liver compared to control. The full gizzard of broilers in R2 was statistically heavier ($p<0.001$) than in R3. As for the non-edible part of carcasses, broilers fed with the R1 diet had significantly heavier ($p<0.05$) head weights compared to the control. In contrast, the

Table 1: Effects of the betel and galangal powders combination as feed additives on the growth performance in broilers

Parameter	Additive				*p*-Value
	BC	R1	R2	R3	
1 -21 days					
Final BW (g)	941.40 ± 16.84	924.13 ± 19.03	924.80 ± 12.07	924.60 ± 25.56	0.90
BWG (g)	886.40 ± 16.61	872.20 ± 18.25	869.07 ± 11.94	872.07 ± 25.44	0.87
Cumulative FCR	1.31 ± 0.02	1.33 ± 0.03	1.33 ± 0.02	1.34 ± 0.04	0.87
22 -35 days					
Final BW (g)	2338.07 ± 47.51	2423.93 ± 65.83	2385.67 ± 42.87	2372.27 ± 77.58	0.79
BWG (g)	1396.67 ± 36.69	1499.80 ± 49.34	1460.87 ± 34.08	1447.67 ± 57.72	0.40
Cumulative FCR	1.49 ± 0.04	1.40 ± 0.05	1.42 ± 0.03	1.45 ± 0.06	0.49
1 -35 days					
Final BW (g)	2338.07 ± 47.51	2423.93 ± 65.83	2385.67 ± 42.87	2372.27 ± 77.58	0.79
BWG (g)	2283.07 ± 47.64	2372.00 ± 65.10	2329.93 ± 42.81	2319.73 ± 77.56	0.77
Cumulative FCR	1.41 ± 0.03	1.37 ± 0.04	1.38 ± 0.02	1.40 ± 0.05	0.81

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at p< 0.05

neck weights of all the experimental groups were significantly lower ($p<0.001$) than the control group. R1 carcasses had significantly heavier ($p<0.001$) proventriculus than control. R1 and R2 carcasses possessed significantly heavier ($p<0.001$) GIT weight than control. In summary, R1 and R2 broilers showed more carcass characteristics improvements in significantly higher dressing percentage and higher edible giblets weights compared to control. As for R3 broilers, no significant improvements were observed in dressing percentage and edible giblets compared to control.

3.3 Histomorphometry of Villi and Tunica Muscularis in Small Intestine

3.3.1 Duodenum

The effects of betel and galangal powder as feed additives on duodenal histomorphometry

Table 2: Effects of betel and galangal powders combination as feed additives on the carcass quality in broilers

Parameter	Additive				*p*-value
	BC	R1	R2	R3	
Final live weight (g)	2338.07 ± 47.51	2423.93 ± 65.83	2385.67 ± 42.87	2372.27 ± 77.58	0.79
Kill-out weight (g)	2146.73 ± 44.69	2281.93 ± 60.64	2278.33 ± 50.86	2175.13 ± 64.76	0.21
De-feathered weight (g)	2022.87 ± 44.10	2164.73 ± 57.94	2134.93 ± 43.05	2053.20 ± 65.12	0.21
Dressing percentage (%)	71.54^{b} ± 0.85	75.12^{a} ± 0.63	74.63^{a} ± 0.23	71.24^{b} ± 1.36	**<0.001**
Breast (g)	649.60 ± 17.44	713.47 ± 24.13	714.27 ± 16.21	697.07 ± 24.68	0.11
Drumstick (g)	222.47^{ab} ± 6.60	216.00^{b} ± 6.70	222.73^{ab} ± 6.51	244.87^{a} ± 8.92	**0.04**
Wing (g)	170.27^{ab} ± 5.59	155.60^{ab} ± 6.10	177.13^{a} ± 12.61	140.93^{b} ± 11.18	**0.04**
Head (g)	60.40^{b} ± 1.98	70.85^{a} ± 2.46	58.17^{b} ± 1.70	61.09^{b} ± 2.72	**0.01**
Neck (g)	167.07^{a} ± 13.03	59.33^{b} ± 9.74	46.47^{b} ± 3.41	39.07^{b} ± 1.99	**<0.001**
Shanks (g)	89.40 ± 3.99	82.00 ± 2.98	76.47 ± 3.17	78.27 ± 4.04	0.06
Heart (g)	9.33^{b} ± 0.19	13.33^{a} ± 0.78	10.73^{ab} ± 0.31	10.50^{b} ± 0.59	**<0.001**
Liver (g)	40.21^{c} ± 1.15	48.53^{a} ± 1.94	46.59^{ab} ± 1.11	41.43^{bc} ± 1.50	**<0.001**
Full gizzard (g)	30.83^{ab} ± 2.37	37.20^{ab} ± 1.85	37.37^{a} ± 1.44	29.48^{b} ± 2.48	**0.01**
Empty gizzard (g)	29.05 ± 2.12	33.04 ± 1.59	30.45 ± 1.02	27.39 ± 2.24	0.08
Proventriculus (g)	7.84^{b} ± 0.29	9.87^{a} ± 0.61	9.24^{ab} ± 0.44	7.68^{b} ± 0.28	**<0.001**
GIT (g)	61.69^{b} ± 2.10	97.53^{a} ± 3.63	101.60^{a} ± 3.06	68.90^{b} ± 2.30	**<0.001**

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at p< 0.05

from the broilers in the experiment are shown in Table 3. For the duodenal villi morphometry, the villi heights of broilers in the R1 group were significantly higher ($p<0.001$) than the control group, R2 and R3. Meanwhile, the villi widths of broilers in the R2 group were significantly wider ($p<0.001$) than the control group and R1. Regarding crypt depth, broilers in the R3 group had significantly deeper ($p<0.001$) crypts than the control. However, the villi height to crypt depth ratios of all groups showed no significant difference ($p>0.05$) among each other. All experimental groups had significantly higher ($p>0.05$) values for villi surface areas than control, with the best villi surface areas from the R2 group. The broilers in the R1 group had significantly thicker ($p<0.001$) duodenal tunica muscularis than control in the experiment.

In brief, R1 and R2 broilers showed better modulation in duodenal villi histomorphometry than R3 broilers. R1 broilers had the highest villi, shallowest crypt depth and thickest tunica muscularis; meanwhile, R2 broilers had the widest villi, highest villi height to crypt depth ratio and largest villi surface area. The representative histological sections of the duodenum from each group are shown in Appendix Fig. 4.

Table 3: Effects of betel and galangal powders combination as feed additives on the histomorphometry of the duodenum in broilers

Parameter	Additive				*p*-Value
	BC	R1	R2	R3	
	Duodenum				
Villi height (µm)	$1028.01^{b} \pm 8.80$	$1160.04^{a} \pm 4.77$	$1050.00^{b} \pm 8.54$	$1008.95^{b} \pm 12.50$	**<0.001**
Villi width (µm)	$130.08^{b} \pm 4.00$	$128.71^{b} \pm 5.70$	$156.81^{a} \pm 6.33$	$141.83^{ab} \pm 6.39$	**<0.001**
Crypt depth (µm)	$149.24^{b} \pm 4.16$	$156.18^{ab} \pm 7.62$	$162.18^{ab} \pm 14.33$	$173.86^{a} \pm 4.41$	**<0.001**
Villi height: Crypt depth	7.24 ± 0.26	7.28 ± 0.44	9.45 ± 1.35	6.52 ± 0.36	0.06
Villi Surface Area ($\times 10^{-s}$ µm²)	$413.49^{b} \pm 7.51$	$474.97^{c} \pm 8.66$	$533.01^{a} \pm 6.53$	$469.48^{c} \pm 7.84$	**<0.001**
Tunica muscularis thickness (µm)	$145.38^{b} \pm 10.17$	$221.15^{a} \pm 11.80$	$167.50^{ab} \pm 14.23$	$169.70^{ab} \pm 18.71$	**<0.001**

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at $p< 0.05$

3.3.2 Jejunum

The effects of betel and galangal powders as feed additives on jejunal histomorphometry from the broilers in the experiment are shown in Table 4. Regarding jejunal villi morphometry, the

Table 4: Effects of betel and galangal powders combination as feed additives on the histomorphometry of the jejunum in broilers

Parameter	Additive				*p*-Value
	BC	R1	R2	R3	
	Jejunum				
Villi height (µm)	$806.23^{bc} \pm 7.14$	$734.44^{b} \pm 17.95$	$919.41^{ac} \pm 19.39$	$946.09^{a} \pm 7.94$	<0.001
Villi width (µm)	$111.47^{b} \pm 3.59$	$106.23^{b} \pm 6.71$	$139.48^{a} \pm 5.66$	$116.14^{ab} \pm 4.72$	<0.001
Crypt depth (µm)	$149.40^{c} \pm 5.31$	$117.13^{b} \pm 4.26$	$181.24^{a} \pm 5.21$	$143.50^{c} \pm 3.63$	<0.001
Villi height: Crypt depth	5.38 ± 0.45	5.78 ± 0.73	5.23 ± 0.23	6.61 ± 0.44	0.13
Villi Surface Area ($\times 10^{-3}$ µm^2)	$285.01^{bc} \pm 13.00$	$250.51^{b} \pm 22.59$	$392.33^{a} \pm 23.43$	$355.29^{ac} \pm 22.86$	<0.001
Tunica muscularis thickness (µm)	164.53 ± 4.37	147.67 ± 5.21	160.88 ± 5.30	159.05 ± 5.08	0.24

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at $p < 0.05$

broilers in the R3 group had significantly higher ($p<0.001$) villi heights than both the control and R1 groups, whereas R2 broilers had significantly higher ($p<0.001$) villi heights than the R1 group only. As for the villi width, R2 broilers had significantly wider ($p<0.001$) villi than the control and R1 groups. Different outcomes were recorded for the crypt depth, where the R1 broilers had significantly shallower ($p<0.001$) crypt depths compared to all the groups, and R2 broilers had significantly deeper ($p<0.001$) crypt depths compared to all groups. In regard to the villi surface area, R2 broilers had significantly ($p<0.001$) larger surface areas than both control and R1; meanwhile, R3 broilers had significantly larger ($p<0.001$) surface areas than R1 only. However, the villi height to crypt depth ratios for all groups showed no significant difference ($p>0.05$), similar to the tunica muscularis thickness.

In brief, the R2 broilers showed the best modulation in jejunal villi histomorphometry, followed by the R3 broilers. R2 broilers had the widest villi, largest villi surface area and thickest tunica muscularis; meanwhile, R3 broilers had the highest villi height and the highest ratio of villi height to crypt depth. However, R1 broilers had the best crypt depth, which was the shallowest. The representative histological sections of the jejunum from each group are shown in Appendix Fig. 5.

3.3.3 Ileum

The effects of betel and galangal powder as feed additives on ileal histomorphometry from the broilers in the experiment are shown in Table 5. The R2 diet had the best effect on the villi heights for the ileum compared to other groups and was significantly higher ($p<0.05$) than the R3 group. In addition, the R2 diet had the widest villi and was significantly wider ($p<0.001$) than the control and R1 groups. There were no significant differences ($p>0.05$) recorded for other parameters in the ileum.

In brief, R2 broilers had the best modulation in the ileal villi histomorphometry, followed by R3 broilers. R2 broilers had the highest and widest villi, largest villi surface area and thickest tunica muscularis. On the other hand, R3 broilers had the shallowest crypt and the highest ratio of villi height and crypt depth. The representative histological sections of the ileum from each group are shown in Appendix Fig. 6.

Table 5: Effects of betel and galangal powders combination as feed additives on the histomorphometry of the ileum in broilers

Parameter	Additive				*p*-value
	BC	R1	R2	R3	
Ileum					
Villi height (µm)	$608.96^{ab} \pm 17.15$	$680.46^{ab} \pm 53.49$	$695.76^{a} \pm 60.69$	$564.95^{b} \pm 28.47$	**<0.05**
Villi width (µm)	$103.41^{b} \pm 2.99$	$95.36^{b} \pm 4.27$	$124.53^{a} \pm 6.05$	$110.09^{ab} \pm 4.14$	**<0.001**
Crypt depth (µm)	100.44 ± 2.18	99.95 ± 5.87	109.94 ± 5.22	98.77 ± 2.41	0.14
Villi height: Crypt depth	5.73 ± 0.23	4.74 ± 0.33	5.52 ± 0.63	5.60 ± 0.30	0.06
Villi Surface Area ($\times 10^{-3}$ µm²)	199.80 ± 8.57	176.71 ± 16.56	276.85 ± 29.50	201.76 ± 14.68	0.09
Tunica muscularis thickness (µm)	197.33 ± 6.68	213.40 ± 14.99	216.61 ± 23.96	203.67 ± 9.22	0.31

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at p< 0.05

3.3.4 Association of Villi Histomorphometry with Broiler Performance

The association between the histo-morphometry of villi and broiler performance was

investigated. Based on the significant differences observed compared to the control group, the surface areas of duodenal and jejunal villi were selected as parameters for assessing villi histomorphometry. Similarly, the dressing percentage was chosen as the indicator of broiler performance due to its significant differences compared to the control group. The associations were determined using the correlation coefficient and shown in Table 6. A significantly positive correlation ($p<0.001$; $r = 0.42$) was found between the duodenal villi surface area results and the carcass dressing percentage. On the contrary, the association between the jejunal villi surface area and the carcass dressing percentage was negatively correlated and not statistically significant ($p>0.05$; $r= -0.06$).

Table 6: Correlations between villi surface area in duodenum and jejunum and dressing percentage in broilers

Parameter	Correlation Coefficent Value (*r*)	*p*-Value
Duodenum		
Villi Surface Area (× 10^{-3} µm²) - Dressing Percentage (%)	0.42	<0.001*
Jejunum		
Villi Surface Area (× 10^{-3} µm²) - Dressing Percentage (%)	-0.06	0.41

p-Value with an asterisk superscript is significant at $p< 0.05$

3.4 Histomorphometry of Goblet Cells in Small Intestine

The effects of betel and galangal powders as feed additives on the goblet cell counts and mucin types in the small intestine from the broilers in the experiment are shown in Table 7.

In regard to the comparison of small intestinal goblet cell counts and mucin types, the number of mixed mucin goblet cells in the duodenum in the R1 and R2 groups was significantly higher ($p<0.001$) than in R3. The neutral mucin goblet cells in the jejunum of the R2 broilers were significantly less ($p<0.001$) than the control. As for he ileum, the mixed mucin goblet cell counts in the R2 and R3 groups were significantly higher ($p<0.001$) than in the R1 group. Besides, the total goblet cell counts of ileum in R2 were significantly higher ($p<0.001$) than in the R1 group.

In overview, none of the experimental groups significantly increased the counts of goblet cells regardless of mucin types compared to the control. The current study noted higher counts of acidic and mixed mucins than neutral mucins in the duodenum, jejunum and ileum in all groups. The representative histological sections of the duodenum, jejunum and ileum from each group are shown in Appendix Fig. 7, Appendix Fig. 8 and Appendix Fig. 9 respectively.

4. Discussion

4.1 Assessment of Growth Performance

Various literatures have used the FCR and BWG as metrics to evaluate the broiler performance when investigating the effects of betel and galangal additives [13] [14] [15] [16] [18] [19]. The FCR measures feed efficiency with lower values indicating higher efficiency [31]. Notably, another study on galangal reported significantly lower feed consumption in experimental chickens, resulting in improved FCR, with the best FCR achieved at inclusion levels of 0.25% and 1.00% [13]. Similarly, the highest dosage of galangal at 750 mg/kg in broilers showed the lowest mortality rate, best FCR and BWG [18]. For betel, other

Table 7: Effects of betel and galangal powders combination as feed additives on the goblet cell counts and mucin types in broilers

Parameter	Additive				P Value
	BC	R1	R2	R3	
Duodenum					
Acidic mucin	57.56 ± 4.32	56.65 ± 5.58	63.11 ± 11.24	84.11 ± 14.18	0.37
Neutral mucin	16.89 ± 3.52	17.59 ± 3.42	8.78 ± 1.81	8.22 ± 1.44	0.10
Mixed mucin	32.22^{ab} ± 5.78	54.53^{a} ± 11.98	61.56^{a} ± 12.40	11.33^{b} ± 1.83	**<0.001**
Total cell count	106.67 ± 11.48	128.76 ± 17.28	133.44 ± 10.88	103.67 ± 14.81	0.47
Jejunum					
Acidic mucin	53.67 ± 10.17	58.40 ± 8.90	98.00 ± 21.74	85.33 ± 12.38	0.13
Neutral mucin	37.67^{a} ± 7.29	19.60^{ab} ± 4.53	7.11^{b} ± 2.43	17.22^{ab} ± 2.76	**<0.001**
Mixed mucin	59.00 ± 15.93	34.70 ± 4.18	52.22 ± 14.36	31.33 ± 3.66	0.48
Total cell count	150.33 ± 25.00	112.70 ± 12.91	157.33 ± 37.27	133.89 ± 14.52	0.55
Ileum					
Acidic mucin	96.67 ± 10.50	74.60 ± 11.88	81.89 ± 11.56	91.33 ± 11.24	0.52
Neutral mucin	25.11 ± 5.84	12.00 ± 3.62	21.89 ± 5.71	26.22 ± 4.11	0.16
Mixed mucin	32.00^{ab} ± 4.59	19.00^{b} ± 4.14	82.89^{a} ± 16.00	54.22^{a} ± 10.50	**<0.001**
Total cell count	153.78^{ab} ± 17.00	105.60^{b} ± 17.34	186.67^{a} ± 21.58	171.78^{ab} ± 22.91	**0.03**

Note: **BC**=(Baseline control), **R1**=(0.50% betel, 1.50% galangal), **R2**=(1.00% betel, 1.00% galangal), **R3**=(1.50% betel, 0.50% galangal)

Means with different superscripts in the same row differ significantly at $p < 0.05$

researches have yielded consistent positive results, demonstrating improved FCR and BWG in experimental broilers [14] [15] [16] [19]. The absence of significant difference in growth performance of this study might be due to confounding factors. Possible factors that have been reported as most influenced in broiler performance are temperature, ventilation rate and genetic strain [32]. Study differences in terms of feed availability could also contribute to the differences in the growth performance outcome. In this study, the feeding was not done ad libitum that might limit the potential to significantly increase the FI of the experimental broilers as contrast to other studies [13][14][15].

This study represents the first investigation into the effects of betel and galangal combinations at different ratios, specifically at a 2% inclusion level, on BWG and FCR. Enhanced production parameters in broilers, such as FCR and BWG, have been attributed to the improved small intestinal morphometry resulting from dietary flavonoid contents in the PFAs [33]. Flavonoids modulate small intestinal morphometry by preventing injuries caused by intestinal pathogens through their antibacterial activity, enhancing the growth and regeneration of intestinal villi to increase absorption capacity, and stimulating mucus secretion, ultimately leading to improved villi protection and the growth of probiotic bacteria [33].

4.2 Assessment of Carcass Quality

Ideal broilers should possess high slaughter yields, desirable carcass conformation scores, proper aesthetic, nutritional and healthy characteristics [34]. Broiler selection progress throughout the years emphasized BW increase, improved carcass composition, shorter production period and higher carcass dressing percentage, which depends on more edible portions and less inedible components or slaughter offal [35]. Every group in this experiment achieved final live weight that surpasses the standard of Ross 308 broilers at 35 days of age which is 2296 g [22]. Enhanced carcass weight observed in phytobiotic administration could be because of the proven antimicrobial and antioxidant properties of the saponin content [23]. Additionally, steroid saponin is associated with positive performance parameters in broilers due to its advantages in gut histomorphometry and nutrient absorption [23].

There are associations between BW and carcass weight, similar to carcass weight and dressing percentage [23]. The standard dressing percentage for Ross 308 broilers is 72.38% for male and 72.92% for female at 2.4 kg live weight [22]. Both R1 and R2 broilers had surpassed the standard, however the dressing percentages for BC and R3 broilers were lower than the standard. The values obtained from this study did not deviate far from the reported values in other galangal studies in broilers. Galangal additives enhanced carcass percentage at 7.7%, 6.8%, 3.8%, and 3.2% increases in 0.25%, 0.50%, 0.75%, and 1.00% inclusion levels [13]. In agreement, galangal rhizome extract at 250 mg/kg, 500 mg/kg and 750 mg/kg had been reported to significantly increase the dressing by 1.61%, 3.26%, and 5.6%, respectively [18]. A study revealed administering betel leaf water at 4% level significantly increased carcass weight, but no significant change was found for dressing percentage [17]. Meanwhile, no significant effect was observed on dressing percentage in broilers fed with betel soilage [16].

In poultry, edible parts include lean meat (muscle tissue inclusive of intermuscular fat), skin with subcutaneous fats and giblets (gizzard, liver and heart) [35]. Muscle tissues are the most valuable edible components, with faster growth rates than internal organs and other non-edibles like feathers, blood and head [35]. To compare with the commercial standard, all the groups exceeded the standard breast weight percentage for Ross 308 broilers which is 591.12 g and 638.64 g for male and female respectively at 2.4 kg live weight [22]. The standard drumstick weight for commercial Ross 308 broilers is 236.16 g for male and 222.24 g for female at 2.4 kg live weight [22]. Only the R3 group achieved the standard drumstick weight. None of the groups achieved the standard wing weight for Ross 308 broilers which were 181.2 g for male and 180.48 g for female at 2.4 kg live weight [22]. Broilers fed with galangal additives in another study showed significantly increased dressing, breast and thigh percentages compared to control broilers [18]. Moreover, plant extracts like Yucca schidigera as broiler feed additives increased the breast muscle percentage of the eviscerated carcass by 1.2% [23]. Positive effects observed in breast and thigh meat yield could be associated with saponin content [23]. On the contrary, no significant changes were found in meat, fat and skin percentages, the weight of bones and the weight of meat in betel-fed broilers [17]. Likewise, limited effects on carcass and organ composition of broilers were reported in various dietary flavonoids and herbal additives studies except for the liver [33].

Regarding edible giblets in this study, another study in broiler fed with galangal reported significantly increased liver, heart and gizzard percentages [18]. Contrarily, no significant effect was observed on liver and pancreas weights in broilers fed with betel soilage [16]. A meta-analysis study found that flavonoid and herbal additives in broilers typically increased liver weight, suggesting a potential detoxification process occurring in the liver [33]. Regarding gizzard, heavier gizzard weights in broilers were strongly correlated with better feed efficiency [31].

Non-edibles comprised of bones and slaughter offals that include blood, feathers, head, feet, GIT with the digesta and peri intestinal fat, abdominal fat, trachea, lungs and reproductive organs, pancreas, spleen and kidneys [35]. The neck weight results in this study were in contrast to another phytobiotic additive, Yucca schidigera, which revealed a significant neck weight increase

in the highest dosage but still no significant changes on other non-edibles like shank and GIT [23]. Although GIT weight increases were considered negative in terms of edibility, they were beneficial in terms of feed efficiency. The current study was the first to evaluate the effects of betel and galangal powders combination on inedible portions of carcass characteristics.

The findings in this study showed that the feed additives increased the carcass quality to some extent in terms of distribution of the tissue components due to the increase in lean meat on the most valuable carcass parts like breast and drumstick, instead of less valuable wing and neck parts as recommended [35]. Differences from the results of this study and previous experiments could be due to various factors like strains of bird used, the environment, type of feed, source of saponins and the amount of additives applied [23].

4.3 Histomorphometry of Villi and Tunica Muscularis in Small Intestine

Intestinal morphometry is significantly associated with broiler performance under commercial conditions [36]. Enhancing intestinal morphometry and functionality by PFAs will lead to more efficient nutrient uptake for growth and daily physiological processes and functions [37].

In broilers, the duodenum is the major site of digestive enzyme secretion and the beginning of nutrient absorption [35] [38]. The majority of proteins are digested in the duodenum, which amounts to about 60% relative contribution in protein digestion of the chickens, as compared to jejunum and ileum which merely constitute 25% and 8%, respectively [39]. Furthermore, most glucose absorption occurs in the duodenum [40].

The key function of the jejunum is as the site where a large extent of the major nutrients is digested and absorbed [41]. In context, 63% of amino acid absorption occurs in the jejunum, whereas only 7% and 13% are absorbed in the duodenum and ileum, respectively [39]. Also, the highest trypsin and amylase concentrations were observed in the jejunum, possibly due to the pancreatic ducts location that entered nearly at the duodenum end [40]. Due to the fact that dry matter spent 25% of its retention time in the jejunum, the upper or proximal jejunum was the major sodium, potassium, calcium, magnesium and phosphate absorption site [40].

The ileum is a mineral and water absorption site, although some protein and fat digestion and absorption may occur [41]. The ileum had the highest mean feed or digesta retention time at 90 or 97 minutes, followed by the jejunum at 71 or 84 minutes and only 5 or 10 minutes in the duodenum [40]. Plus, most of the ingested starch was digested in the terminal ileum, reaching up to 97%, followed by jejunum and duodenum at 85% and 65%, respectively [40].

Villi height is one factor that can affect animal nutrient uptake. By increasing the height of the villi, more epithelial cells are present for nutrient absorption to be utilized by the animals [37]. The increased height observed in the current experiment is consistent with a study which found that administering the phytogenic blend that included betel significantly increased duodenal and jejunal villi heights [19]. Similarly, 4 g/kg betel additive resulted in maximal duodenal and jejunal villi heights [14] [15]. Curcumin, which is in the same Zingiberaceae family as galangal, was reported to increase the villi heights in the duodenum, jejunum and ileum in experimental broilers at 200 mg/kg feed [42].

The current experiment recorded an increase in villi width together with other parameters. Villi width improvement greatly influenced the increase in intestinal surface area, leading to greater BW in broilers [43]. This idea was agreed upon a study reported that an increment in intestinal width, among other parameters, will facilitate nutrient absorption [44]. Another research observed similar results as this research, which increased the villi width at the duodenum and jejunum by adding curcumin in a broiler diet [42]. One PFA study in broilers involving *Punica granatum*, Thymus vulgaris and Allium sativum only observed improvement in villi width without significant changes in other parameters [44]. Both shallower and deeper crypt depths were observed in this study within the intestines of experimental broilers. Shallower intestinal crypt depths can decrease intestinal mucosal turnover or enterocyte differentiation activity and reducing maintenance

requirements [23]. When villi regeneration requires less maintenance, more energy can be allocated to support growth performance, leading to a higher BW. This is supported by other studies that mentioned betel inclusion in broiler feed resulted in shallower crypt depths in duodenum and ileum [15] and jejunum [19]. Deeper crypts can result in poorer performance and reduced nutrient absorption in broilers due to faster tissue turnover, particularly in situations involving tissue sloughing, inflammation or the presence of toxins [42]. However, some studies reported the increase in crypt depth with feed additive as beneficial to the broilers since deeper crypt allows rapid and larger villi regeneration that enhances nutrient absorption [27] [37]. Nevertheless, when considering the villi height to crypt depth ratio, the shallower crypt depth becomes more applicable due to the favorable higher ratio observed in broilers.

A higher villi height and crypt depth ratio are desired in feed additive study as they produce greater nutrient digestibility and absorption capacity [37]. However, a contrast observation was recorded by a study where no statistically significant changes in villi height to crypt depth ratio was observed in broilers fed with PFA [44]. Another study reported that *Moringa oleifera* leaf meal did not significantly affect the villus height to crypt depth ratio [45]. Nevertheless, all the jejunal ratios recorded in this study (despite one group being lower than the control) were higher than the optimal healthy jejunal villi height to crypt depth ratio of 3.26 [46].

Due to the relatively short, small intestine in broilers, nutrient absorption depends greatly on the villi surface area [43]. A reported PFA study, *Moringa oleifera* leaf meal, increased the jejunal villi surface area at 7.5% broiler feed [45]. Another *Moringa oleifera* leaf meal study at 1.2% also reported significantly increasing the duodenal villi surface area [30]. In agreement, commercial curcumin additive at 50 g per ton basal diet also significantly increased the jejunal villi surface area [47]. The observed histomorphometric changes were more common in the duodenum and jejunum than in the ileum. This might be because these segments play a more significant role in digesting and absorbing nutrients from the feed, including PFA [38] [41].

Tunica muscularis in all the intestinal segments aid in propelling nutrients in a uniform direction from the lumen into the submucosal layer [48]. Increasing muscular layer thickness may increase the contact between intestinal content and mucosa and improve digestive capacity [49]. Thymol, a compound isolated from galangal root in a previous study [50], has been reported to increase the muscular layer thickness of the jejunum and ileum in broilers [51]. A previous study on the rosemary leaf meal inclusion at 5 g/kg broiler feed also revealed thickened duodenal tunica muscularis thickness, with similar findings in jejunum and ileum [48]. Similar observations were noticed at 7.5% M. oleifera additive [45] and 75 g per ton of commercial curcumin and 2.5 kg turmeric powder per ton in the jejunal muscle thickness of broilers [47].

Enhanced epithelial cell proliferation and villi elongation observed in this study could be attributed to the phytochemical constituent in betel and galangal. Flavonoids, present among other compounds in betel and galangal, positively impact the intestinal tract [33]. Quercetin, a type of flavonoid, was established by previous works in betel [52] and galangal [18]. Quercetin was reported to improve the biological development of broilers via growth hormone upregulation with hepatic growth hormone receptors, which consequently leads to higher insulin-like growth factor-1 (IGF-1) concentration [14] [15]. Insulin-like growth factors are expressed in the duodenum, jejunum and ileum, playing crucial roles in gastrointestinal development, even during the early stages of hatchling life [39]. The binding of IGF-1 to its receptors located on the basolateral membrane of intestinal epithelial cells and smooth muscle cells triggers a signaling cascade within the cells [53]. This cascade ultimately stimulates intestinal epithelial cell and muscle cell proliferation and maintenance of cell survival by reducing apoptosis [53].

The thyroid hormone receptors regulate intestinal epithelium homeostasis that balances cell proliferation and differentiation [54]. Thus, thyroid hormones, such as thyroxine (T4) and

triiodothyronine (T3), can influence intestinal epithelial cell proliferation and villus length. A study reported that galangal treatment significantly increased both triiodothyronine (T3) and thyroxine (T4) levels in broilers, which could influence intestinal epithelial cell proliferation and villi length [13]. Meanwhile, the administration of betel leaf extract in rats was found to decrease T3 concentrations while increasing T4 concentrations at higher doses and it had the opposite effect at lower doses [11]. The effects of T4 on early gastrointestinal development were increased microvilli growth, higher rates of epithelial mitosis and elevated active glucose transport [39].

Besides, the elongated and wider villi that were observed in this study could be due to the affected intestinal stem cells (ISC) located within the crypts that regulate the villi growth. The ISC possesses two crucial intestinal epithelium homeostasis characteristics [55]. Firstly, the self-renewal properties of the stem cells that are able to replicate rapidly and continuously (proliferation). Next, the multipotency trait transforms into differentiated cells that include enterocytes, goblet cells, enteroendocrine cells and Paneth cells (differentiation).

Several phytochemical compounds in different classes were reported to influence stem cell proliferation, differentiation or both proliferation and differentiation [56]. The first class is the phenolic compounds. Ferulic acid which also affects the proliferation was previously detected in betel [11] [19] [57] and galangal [12] [58]. Anthocyanin, observed to improve differentiation, was found among galangal constituents [12] [13] [58]. Next, another phenolic compound, piceatannol, which stimulated the differentiation, was reported as a phytochemical constituent in betel [11] [57]. Curcumin, which modulated both proliferation and differentiation, was identified in galangal [12] [59].

For flavonoid compounds, apigenin plays a role in the differentiation of stem cells and was recorded as the phytochemical compound in galangal [12] [18] [58]. Additionally, the differentiation was also influenced by chrysin, which was found in both betel and galangal studies [11] [2] [57] [58]. Plus, quercetin, which modulated both proliferation and differentiation, was isolated in betel and galangal [14] [15] [18] [19] [58].

Lastly, daucosterol is a type of sterol that helps in the proliferation of stem cells and is available in the betel stem [11]. Therefore, these various phytochemical compounds in betel and galangal may influence the proliferation and differentiation of ISC, consequently resulting in the villi morphometric changes observed in this study.

4.4 Histomorphometry of Goblet Cells in Small Intestine

Goblet cells dispersed among the intestinal epithelial cells play a role in synthesizing and secreting mucins within the intestinal tract, which is influenced by immune system regulation [60]. Mucins that form the intestinal mucus layer facilitate the lubrication process in food passage, participate in cell signaling pathways and protect the epithelium against commensal microbes, pathogen invasions, toxins and other environmental irritants [60]. Alteration in mucin quantity and composition at the intestinal surface of the mucosa can lower nutrient absorption and/or increase the energy requirement for intestinal function maintenance [37].

A study on the impact of *Moringa oleifera* leaf powder additive in broilers reported that the total goblet cell count was higher in all the experimental groups [30]. Another study also reported that feed additives like prebiotics and probiotics maintained the enhanced activity of goblet cells in the small intestine of the broilers in heat-stress conditions [61].

In terms of physiological relevance, it is not well understood why mucins have distinct types, acidic or neutral. However, it is suggested that it might be related to their protective characteristics [62] [63]. Distribution of different mucin types in goblet cells is affected by various factors within the animals themselves, such as inflammatory markers, hormones and neurotransmitters, and also external factors like commensal bacteria, pathogens, pre/ probiotics and nutrients in the diet [62].

The current study noted higher counts of acidic mucins and mixed mucins compared to neutral mucins in the duodenum, jejunum and ileum in all groups. This observation is similar to the findings that reported higher counts of acidic mucin in the duodenum, jejunum and ileum in the group with 1.2% *Moringa oleifera* additive [30]. In addition, mixed goblet cells are commonly observed in the small intestine of chickens in normal conditions [62].

Acidic mucins have been reported as advantageous against bacterial invasion due to the possession of terminal ends of O-glycan with O-acetylated sialic acid (sialomucins) or sulfated group (sulfomucins), that protect the mucin chains from degradation by bacterial enzymes such as proteases and glycosides and proteolytic host enzymes [62] [63]. This also explains why acidic mucins have been found in intestinal regions densely populated by microbes [63]. In stressful conditions such as heat stress, the broiler intestine goblet cells increase in number and increase in the maturation process cycle, where the mucin composition gradually changes from neutral to acidic [61].

5. Conclusion

The dressing percentage of the R1 group significantly increased at 3.58%, while the R2 group significantly increased at 3.09% compared to the baseline dressing percentage from control group.

Numerous positive histomorphometric responses were observed in the small intestine that could indicate improvement of digestion and nutrient absorption in the study. The additives positively affected the duodenum, jejunum and ileum in terms of villi height, width, crypt depth, tunica muscularis thickness and villi surface area. Villi height to crypt depth ratio showed no significant difference on intestinal segments. There was no significant difference in goblet cells and mucin in all the experimental groups compared to control except decreased jejunal neutral mucin at the expense of higher acidic mucins. The current study noted higher counts of acidic and mixed mucins than neutral mucins in the duodenum, jejunum and ileum in all groups. Acidic mucins have been reported as advantageous against bacterial invasion.

In conclusion, the addition of betel and galangal powder at 2% of broiler feed improves both carcass characteristics and small intestine histomorphometry. The improvements observed through the histological perspective at the cellular level in the experimental groups were reflected in the carcass characteristics. Based on the evidence and results from the carcass characteristics and histomorphometric responses of the small intestine from this in-vivo study, the betel and galangal powders combination has the potential to be utilized as polyherbal feed additives to improve the broiler production in the future.

Availability of Data and Materials

All data are available in this study

Author Contributions

Conceptualization, [MAIMK], [MFHR] and [MAMN]; Methodology, [MAIMK], [MAMN], [AFMA], [II], [MRHR], [BSG], [FFAH] and [SHS]; Validation, [MAIMK], [MAMN] and [SHS]; Formal Analysis [MAIMK], [MAMN] and [AFMA]; Investigation, [MAIMK] and [SNAM]; Resources, [MFHR] and [LA]; Writing - Original Draft, [MAIMK]; Writing - Review & Editing, [MAIMK], [MFHR], and [LA].

Ethics Approval and Consent to Participate

The animal experiment was conducted following the Ethical Committee for the Experimental Use of Animals at the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan.

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Conflict of Interest

The authors declare no conflict of interest.

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