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Abstract

This study evaluated the effects of dietary cabbage by-product powder (CBP) supplementation on growth performance, meat quality, relative organ weight, liver characteristics, welfare, antioxidant capacity, and intestinal barrier function in broiler chickens. A total of 126 one-d-old mixed-sex Ross 308 broiler chickens were randomly allotted to 3 dietary treatments with 7 replicates of 6 birds per cage. Birds were fed a basal diet without CBP supplementation or diets supplemented with 0.05 or 0.10% CBP. The CBP was prepared by drying and grinding the remaining solid residue after the hot-water extraction of cabbage. Experimental diets and water were provided ad libitum for 35 d. Dietary CBP supplementation did not affect growth performance, breast meat quality, relative organ weight, serum parameters, liver characteristics, welfare, jejunal antioxidant capacity, or jejunal morphology. Birds fed diets supplemented with 0.10% CBP had greater ($p < 0.05$) jejunal transepithelial electrical resistance (TEER) than those fed the basal diet or diet supplemented with 0.05% CBP, and TEER showed significant linear and quadratic responses ($p < 0.05$) to increasing dietary CBP levels. The expression of claudin-1 (*CLDN1*) increased linearly ($p < 0.05$) with increasing dietary CBP levels, whereas the expression of occludin and zonula occludens-1 was not affected significantly. In conclusion, dietary CBP supplementation at levels up to 0.10% did not significantly affect growth performance or most other measured outcomes in broiler chickens. However, supplementation with 0.10% CBP increased jejunal TEER, indicating greater jejunal epithelial electrical resistance. These findings suggest that CBP may have potential as a feed material for supporting intestinal barrier function in broiler chickens, although further studies are needed to clarify the underlying mechanisms and physiological significance of the observed TEER response.

Keywords: Broiler chicken; Cabbage by-product; Growth performance; Intestinal barrier function; Tight junction

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INTRODUCTION

Agri-food processing generates substantial amounts of by-products that require effective management and utilization [1]. Although these materials are often excluded from the human food chain, many retain nutrients and other potentially valuable constituents [1,2]. Their use as animal feed may enable the recovery of otherwise underutilized nutrients and biomass while reducing reliance on conventional feed resources and competition between feed and food uses [2]. Accordingly, the utilization of agri-food by-products as animal feed resources has received increasing attention as a strategy for improving resource efficiency in livestock production [3].

Plant-derived by-products may contain dietary fiber, residual nutrients, and various phytochemical compounds, depending on their botanical origin and processing conditions [4]. These characteristics have encouraged their evaluation as alternative feed ingredients or dietary supplements [2]. However, the composition and feed value can vary considerably depending on the plant source and processing method. Therefore, both the origin and processing conditions of the individual residues should be considered when evaluating their potential use in feed diets.

Among plant-derived by-products, cabbage-derived residues represent a substantial source of residual plant material. Cabbage processing generates various fractions, including outer leaves, stems, and cores [5]. These residues may retain dietary fiber, minerals, glucosinolates, phenolic compounds, and other plant constituents, supporting their potential for value-added utilization [5]. However, the composition and functional characteristics of cabbage-derived residues can vary according to the plant fraction and the processing conditions used to produce them. Previous studies have evaluated dried cabbage leaf residues as dietary ingredients in broiler chickens, examining growth performance, nutrient digestibility, intestinal morphology, and blood-related parameters [6-8]. In these studies, cabbage leaf residues were processed through drying and size reduction without prior hot-water extraction. In contrast, the cabbage by-product powder (CBP) used in the present study was obtained from the solid residue remaining after hot-water extraction of cabbage, followed by pressing, drying, and grinding. This distinction is important because hot-water processing may alter the composition of the remaining solid materials through leaching and thermal degradation [9,10]. Consequently, findings obtained using conventionally dried cabbage leaf residues may not be directly applicable to this extraction-derived CBP. In addition, previous studies have shown that plant-derived dietary components can influence intestinal physiology in poultry. Dietary fiber may affect intestinal function through microbial fermentation and the resulting metabolites [11], whereas phytogenic feed additives have been associated with changes in intestinal epithelial function and barrier-related

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responses in non-ruminant animals [12]. Given the limited information available on the dietary use of this hot-water extraction-derived CBP in broiler chickens, low inclusion levels were selected for an initial exploratory evaluation rather than as a substantial replacement for conventional feed ingredients. However, whether supplementation with this CBP at low inclusion levels affects growth performance and other physiological characteristics in broiler chickens remains unclear. Accordingly, this study aimed to evaluate the effects of dietary CBP supplementation on growth performance, meat quality, relative organ weight, liver characteristics, welfare, antioxidant capacity, and intestinal barrier function in broiler chickens.

MATERIALS AND METHODS

Diets, animals, and experimental design

A total of 126 one-d-old mixed-sex Ross 308 broiler chickens (63 males and 63 females) with an average initial body weight (BW) of 44.6 ± 0.08 g were obtained from a local commercial hatchery (Dongsan Hatchery) and randomly allotted to 1 of 3 dietary treatments in a completely randomized design. Each treatment consisted of 7 replicate cages with 6 birds per cage (3 males and 3 females). Birds were housed in four-tier battery cages (52 × 61 × 42 cm; width × length × height) and managed according to the recommendations for Ross 308 broiler chickens throughout the 35-d experimental period [13]. Dietary treatments were randomly assigned to replicate cages across all available cage positions without stratification by battery tier. The basal diets were formulated to meet or exceed the nutritional specifications for Ross 308 broiler chickens (Table 1) [13]. The CBP used in the experimental diets was obtained from the solid residue remaining after hot-water extraction of whole cabbage heads, including outer leaves, inner leaves, and cores, cultivated in Jeju Island, Republic of Korea. A single batch of CBP was used throughout the experiment. Briefly, cabbage and purified water were mixed at a ratio of 30:70 (w/w) and extracted at 101°C for 2 h. After stopping the steam supply, the mixture was allowed to stand in the extraction vessel for an additional 10 h without further heating. The remaining solid cabbage residue was pressed to remove excess moisture, resulting in a moisture content of approximately 85%. The pressed cabbage by-product was dried at 85°C for 24 ± 2 h until the moisture content was below 10%. The final CBP had a dry matter content of 98.62%. The dried cabbage was ground to approximately 60 mesh using a pin mill. The resulting CBP was stored at ≤10°C until use. The proximate composition of CBP was analyzed according to the AOAC [14]. The total phenolic content, total flavonoid content, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity were determined as described by Ku et al. [15]. The chemical compositions and antioxidant characteristics of the CBP

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are presented in Table 2. Dietary treatments consisted of a basal diet without CBP supplementation and the basal diet supplemented with 0.05 or 0.10% CBP. Given the limited information available on the dietary use of hot-water extraction-derived CBP in broiler chickens, these inclusion levels were selected for an exploratory evaluation of low-level CBP supplementation. For the supplemented diets, CBP was initially mixed with a small amount of the basal diet to facilitate even distribution and was then incorporated into the remaining diet at the designated inclusion levels, without adjusting for other dietary ingredients. The three-phase feeding program consisted of starter (1 - 7 d), grower (8 - 21 d), and finisher (22 - 35 d) phases. The experimental diets were provided in crumble form during the starter phase (1–7 d) and in pelleted form during the grower (8–21 d) and finisher (22–35 d) phases. Feed and water were provided ad libitum throughout the experiment. A lighting schedule of 23 h light and 1 h dark was maintained. The BW and feed intake (FI) were recorded at the end of each feeding phase. BW gain (BWG) was calculated for each phase, and feed conversion ratio (FCR) was calculated as FI divided by BWG. No mortality or culling occurred during the 35-d experimental period. Therefore, no correction for mortality was applied to the FCR calculation.

Sample collection

At the conclusion of the experiment, two male birds per replicate with a BW close to the mean BW of the corresponding cage were selected and euthanized by CO_2 asphyxiation. Only male birds were selected for postmortem and intestinal analyses to minimize potential sex-related variation in the measured physiological parameters. One bird was used for tissue and blood collection, whereas the other was used exclusively for the assessment of jejunal permeability. Blood was collected via cardiac puncture into a serum separator tube containing clot activator (Greiner Bio-One). The liver, spleen, bursa of Fabricius, thymus, and abdominal fat were excised from the bird used for tissue and blood collection and weighed; their relative weights were expressed as percentages of BW. Breast muscles were excised and weighed to determine the breast yield, and the samples were stored at 4°C until subsequent meat quality analyses. A jejunal segment was collected, gently rinsed with phosphate-buffered saline, and fixed in 10% neutral buffered formalin for subsequent histomorphological analysis. Jejunal mucosa samples were collected and immediately stored at −80°C for subsequent analyses of antioxidant capacity and tight junction-related gene expression.

Breast meat quality

Breast yield was calculated as the percentage of breast muscle weight relative to the BW. Muscle pH

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was measured at 1 and 24 h postmortem using a portable pH meter (HANNA Instruments). The meat color, including CIE L*, a*, and b*, was determined using a spectrocolorimeter (Konica Minolta). Water holding capacity (WHC) was measured at 24 h postmortem according to the method described by Lee et al. [16], with minor modifications. Briefly, 1.5 g of breast meat was placed in a 50-mL conical tube containing Whatman No. 3 filter paper (Whatman) and centrifuged at 3,000 rpm for 15 min at 4°C. The WHC was calculated from the difference in moisture content before and after centrifugation. Lipid oxidation was evaluated at 7 d postmortem by measuring thiobarbituric acid reactive substances (TBARS) according to the method of Lee et al. [17], with minor modifications. Breast meat samples designated for TBARS analysis were placed in individual sample bags and stored at 4°C for 7 d. Briefly, 5 g of breast meat was mixed with 15 mL of deionized distilled water and 50 μL of 0.01% butylated hydroxytoluene in ethanol and homogenized. One milliliter of the homogenate was mixed with 2 mL of a solution containing 0.2883% thiobarbituric acid and 15% trichloroacetic acid. The mixture was vortexed and incubated in a water bath at 90°C for 15 min. After cooling on ice, the samples were centrifuged at 3,000 rpm for 10 min, and the absorbance of the supernatant was measured at 531 nm using a microplate reader (INNO Microplate Spectrophotometer, LTEK Co., Ltd.). TBARS values were calculated by multiplying the absorbance at 531 nm by 5.88 and expressed as mg malondialdehyde (MDA)/kg meat.

Blood parameters

Blood samples were allowed to clot at room temperature for 30 min and centrifuged at 1,800 × g for 10 min. The separated serum was transferred to microcentrifuge tubes and stored at −20°C until analysis. Serum samples were used to evaluate lipid-related biochemical parameters, including total cholesterol, triglycerides, high-density lipoproteins, and low-density lipoproteins. The concentrations of these parameters were determined using a Cobas C702 automatic biochemical analyzer (Roche Diagnostics) and expressed as mg/dL.

Liver characteristics

Liver color was measured immediately after euthanasia using a colorimeter (CR-400, Konica Minolta), and CIE L*, a*, and b* values were recorded. After color measurement, digital images of the liver were obtained for the subsequent assessment of hemorrhagic and fatty liver scores. Digital images were independently evaluated by five trained observers who were blinded to dietary treatments, and the mean score was used for statistical analysis. Liver hemorrhage was scored on a scale of 0 to 5, according to Diaz et al. [18], where 0 indicates a normal liver and 5 represents extensive and severe hemorrhage. Fatty liver appearance was evaluated according

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to Choi et al. [19] using a scale of 1 to 5, with 1 indicating a normal liver and 5 indicating a pale yellowish-red appearance.

Welfare-related indicators

Welfare-related indicators were assessed at the end of the experiment, according to the Welfare Quality® Assessment protocol for poultry [20]. Two birds per replicate were randomly selected, representing one-third of the birds in each cage [21]. The gait score, footpad dermatitis, hock burn, and feather cleanliness were visually evaluated by five trained observers who were blinded to dietary treatments. Gait score was assessed on a 0-to-5 scale, where 0 indicated normal walking ability and 5 indicated severe lameness with an inability to walk. Footpad dermatitis and hock burns were scored on a 0-to-4 scale, with 0 representing no visible lesions and 4 representing severe lesions. Feather cleanliness was evaluated on a 0-to-3 scale, where 0 indicated clean feathers and 3 indicated extensive fecal contamination.

Antioxidant capacity in the jejunum

Jejunal mucosa samples were used to evaluate the antioxidant capacity. MDA and total antioxidant capacity (TAC) were measured using an EZ-Lipid Peroxidation Assay Kit (DG-TBA200, DoGenBio Inc.) and an EZ-Total Antioxidant Capacity Assay Kit (DG-TAC200, DoGenBio Inc.), respectively. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (23225, Thermo Fisher Scientific), and MDA and TAC values were normalized to the corresponding protein concentrations. All assays were performed according to the manufacturer's instructions.

Jejunal morphology

For morphological analysis, an approximately 5-cm jejunal segment was collected from a region 3 - 5 cm proximal to Meckel's diverticulum and gently rinsed with phosphate-buffered saline to remove luminal contents. Jejunal samples were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Paraffin-embedded tissues were sectioned at a thickness of 4 μm and stained with hematoxylin and eosin. The histological sections were examined under a microscope (OS-370DVM, Osun Hitech). The villus height (VH), villus width (VW), and crypt depth (CD) were measured, and the mean of 10 measurements per section was used for subsequent analysis. The VH:CD ratio was calculated by dividing the VH by the CD.

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Jejunal permeability and tight junction-related gene expression

Jejunal permeability was assessed by measuring transepithelial electrical resistance (TEER) using a two-channel Ussing chamber system (P2300, Physiologic Instruments Inc.), as previously described by Kim et al. [22]. Birds were not fasted before tissue collection. A segment from a region 3-5 cm proximal to Meckel's diverticulum was excised immediately after euthanasia and immersed in Krebs–Henseleit buffer (K3703, Sigma-Aldrich). After removal of the luminal contents, the intestinal segment was opened longitudinally, and the mucosal layer was carefully separated from the underlying tissues and mounted as quickly as possible on chamber inserts with an exposed tissue area of 1 cm^2. Before tissue measurements, the resistance of Krebs–Henseleit buffer without tissue was measured as a reference for background resistance correction. The chambers were maintained at 40°C and filled with Krebs–Henseleit buffer, which was continuously aerated with a mixture of 95% O_2 and 5% CO_2. After a 10-min equilibration period following tissue mounting, electrical resistance was recorded at 10-s intervals for 5 min. TEER was calculated by subtracting the background resistance from the measured resistance and multiplying the corrected value by the exposed tissue area (1 cm^2), and was expressed in $\Omega \cdot cm^2$. The mean TEER value recorded over the 5-min measurement period was used for statistical analysis.

The relative mRNA expression of tight junction-related genes in the jejunal mucosa was determined using a QuantStudio™ 1 Real-Time PCR System (Thermo Fisher Scientific). Total RNA was extracted using TRIzol™ Reagent (15596026, Thermo Fisher Scientific) according to the manufacturer's instructions and treated with RNase-free DNase I (EN0521, Thermo Fisher Scientific). RNA purity was assessed by measuring the A260/A280 absorbance ratio using a NanoDrop spectrophotometer (INNO Microplate Spectrophotometer, LTEK). Complementary DNA was synthesized using a RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Fisher Scientific). The target genes were occludin (*OCLN*), claudin-1 (*CLDN1*), and zonula occludens-1 (*ZO-1*). Primers for target gene amplification were designed using the NCBI Primer-BLAST based on published sequences (Table 3). Relative gene expression was calculated using the $2^{\wedge-\Delta\Delta Ct}$ method, with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as the reference gene.

Statistical analysis

Data other than ordinal scoring outcomes were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc.) according to a completely randomized design. Dietary treatment was considered a fixed effect, and each replicate cage served as the experimental unit. Data other than ordinal scoring outcomes were examined for normality and outliers using the UNIVARIATE procedure of SAS [23]. Least squares means were calculated

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using the LSMEANS statement, and the PDIFF option was used for pairwise comparisons when the overall treatment effect was significant. Orthogonal polynomial contrasts were used to evaluate linear and quadratic responses to increasing dietary inclusion levels of CBP for these variables. Ordinal scoring outcomes, including liver hemorrhage score, fatty liver score, gait score, footpad dermatitis, hock burn, and feather cleanliness, were analyzed using the Kruskal–Wallis test (PROC NPAR1WAY) based on cage-level mean scores. For these outcomes, linear and quadratic contrasts were evaluated using PROC GLM after rank transformation with PROC RANK. Statistical significance was set at $p < 0.05$.

RESULTS

Growth performance and breast meat quality

No significant differences in BW, BWG, FI, or FCR were observed among dietary treatments (Table 4). Similarly, breast yield, pH at 1 and 24 h postmortem, meat color (CIE L*, a*, and b*), WHC, and TBARS did not differ significantly among the dietary treatments (Table 5). No significant linear or quadratic responses to increasing dietary CBP levels were observed in growth performance or breast meat quality.

Relative organ weight and blood parameters

There were no significant differences in the relative weights of the liver, spleen, bursa of Fabricius, thymus, or abdominal fat among dietary treatments (Table 6). Total cholesterol, triglyceride, high-density lipoprotein, and low-density lipoprotein concentrations did not differ significantly among treatment groups (Table 7). Neither linear nor quadratic responses to increasing dietary CBP levels were observed for relative organ weight or blood parameters.

Liver characteristics and welfare-related indicators

Dietary CBP supplementation did not significantly affect the liver color (L*, a*, and b*), hemorrhagic score, or fatty liver score (Table 8). Likewise, the gait score, footpad dermatitis, hock burn, and feather cleanliness did not differ significantly among the treatment groups (Table 9). No significant linear or quadratic responses were observed for these variables with increasing dietary CBP levels.

Antioxidant capacity in the jejunum

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Jejunal MDA and TAC concentrations did not differ significantly among the treatment groups (Table 10). No significant linear or quadratic responses were observed for either parameter with increasing dietary CBP levels.

Jejunal morphology

There were no significant differences in VH, VW, CD, or VH:CD ratio among the treatment groups (Table 11). None of the jejunal morphology measurements showed significant linear or quadratic responses to increased dietary CBP levels.

Jejunal permeability and tight junction-related gene expression

Birds fed diets containing 0.10% CBP had greater ($p < 0.05$) TEER values than those fed diets containing 0 or 0.05% CBP (Table 12). Additionally, significant linear ($p = 0.004$) and quadratic ($p = 0.010$) effects in TEER were observed with increasing dietary CBP levels. No significant differences were observed for *OCLN*, *CLDN1*, or *ZO-1* expression. However, *CLDN1* expression showed a significant linear contrast ($p < 0.05$) with increasing dietary CBP levels.

DISCUSSION

Dietary supplementation with CBP at levels up to 0.10% did not significantly affect the BW, BWG, FI, or FCR in broiler chickens. Mustafa and Baurhoo [7] reported no significant effects on overall BWG, FI, or FCR in broiler chickens fed dried cabbage leaf residues at dietary levels of 3 to 9%. Similar results have been reported for *Brassica*-derived residual materials. Hu et al. [24] observed no significant changes in growth performance when broccoli stem and leaf meal were incorporated into broiler diets at levels of 4 to 12%. Although the materials evaluated in these previous studies differed from the hot-water-extracted CBP used in the present study and were included at considerably higher levels, these findings are broadly consistent with the absence of significant changes in growth performance observed in the present study. However, the lack of significant effects on growth performance does not necessarily indicate an absence of physiological responses to dietary supplementation. Intestinal function plays an important role in nutrient digestion and absorption, thereby contributing to the growth performance of broiler chickens [25-27]. The effects of phytogenic feed additives on growth performance have been inconsistent, despite their potential effects on gastrointestinal functions [28]. Thus, changes in specific

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digestive or intestinal processes may occur without measurable changes in the BWG or FCR. Hernández et al. [29] reported that supplementation with botanical extracts containing essential-oil components improved nutrient digestibility in broiler chickens without consistent improvements in overall growth performance. Similarly, Yu et al. [30] reported that dietary trans-anethole, a plant-derived aromatic compound, altered nutrient digestibility and several indices related to intestinal nutrient transport and barrier function without affecting BW, average daily gain, or feed efficiency. These findings support the possibility that digestive or intestinal physiological responses may occur without corresponding changes in growth performance. This interpretation is consistent with the present study, in which dietary CBP supplementation did not significantly affect growth performance, whereas jejunal TEER increased at 0.10% CBP and *CLDN1* expression showed a significant linear response to increasing CBP levels. Therefore, the lack of significant effects on growth performance should not be interpreted as evidence that CBP does not elicit physiological responses.

Dietary CBP supplementation did not significantly affect breast yield, meat pH, meat color, WHC, or TBARS values. Relative organ weight, serum lipid-related parameters, liver characteristics, and welfare scores were also not significantly affected by CBP supplementation. The absence of significant changes in these measurements suggests that dietary CBP at the evaluated levels did not induce broad alterations in the measured physiological characteristics. In addition, no specific physiological or welfare challenges were imposed in the present study, and liver abnormality and welfare scores remained generally low across the treatments. Therefore, these results are better interpreted as showing that CBP supplementation did not produce detectable deterioration in the measured liver and welfare characteristics under the conditions of the present study.

Dietary CBP supplementation did not significantly affect jejunal MDA or TAC levels in broiler chickens. Although the CBP used in the present study exhibited measurable total phenolic content and DPPH radical scavenging activity, these antioxidant-related characteristics were not accompanied by significant changes in jejunal MDA or TAC levels. The dietary inclusion levels of CBP used in the present study may have been insufficient to induce detectable changes in these jejunal antioxidant-related parameters. The absence of an experimentally induced oxidative challenge may also be relevant when interpreting these findings. Cruvinel et al. [31] reported that dietary pequi oil supplementation did not significantly affect serum MDA levels under thermoneutral conditions but linearly decreased these levels under heat stress in broiler chickens. Although the dietary material and sample type differed from those of the present study, these findings suggest that antioxidant-related responses may vary depending on the experimental conditions. Therefore, further studies under oxidative stress conditions are needed to evaluate whether dietary CBP supplementation affects intestinal antioxidant-related

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responses in broiler chickens. Collectively, dietary CBP supplementation at concentrations up to 0.10% did not produce detectable changes in the jejunal oxidative status of broiler chickens.

Dietary CBP supplementation did not significantly affect jejunal VH, VW, CD, or VH:CD ratio in broiler chickens. These histomorphometric indices reflect the structural characteristics of the intestinal mucosa, particularly the villus–crypt architecture [32]. However, the absence of morphological changes does not necessarily imply that other aspects of intestinal function are unaffected. Histomorphometric and electrophysiological measurements are used to evaluate different parameters of the intestinal mucosa [33,34]. Ouyang et al. [35] reported that dietary white grape marc extract did not significantly alter jejunal villus length, CD, or VH:CD ratio in broiler chickens; however, the glucose-induced changes in jejunal tissue conductance measured using an Ussing chamber were significantly affected by supplementation. These findings indicate that dietary interventions may affect intestinal electrophysiological responses without affecting villus-crypt morphology. Accordingly, the lack of morphological changes observed in the present study should be interpreted as the absence of measurable changes in jejunal mucosal architecture rather than as evidence that other functional properties of the intestinal epithelium were unaffected.

Dietary supplementation with 0.10% CBP significantly increased jejunal TEER values in broiler chickens. The TEER reflects the electrical resistance of the intestinal epithelium to ion movement and is used as a functional indicator of intestinal permeability [34]. Therefore, the greater TEER value observed in broiler chickens fed 0.10% CBP indicates increased jejunal epithelial electrical resistance, although this finding alone does not directly demonstrate reduced intestinal permeability. At the 0.10% inclusion level, CBP contributed approximately 1 g/kg diet, with an estimated total phenolic contribution of 12.5 mg GAE/kg diet based on its measured total phenolic content. Given the minimal contribution of CBP to dietary nutrients and fiber at this inclusion level, the observed increase in TEER cannot readily be explained by changes in conventional nutrient supply. However, the limited compositional characterization of CBP prevents attribution of the observed TEER response to specific bioactive constituents, and its biological relevance at such a low inclusion level remains to be established. Additionally, although the overall treatment effect on *CLDN1* expression was not significant, a significant linear contrast was observed with increasing dietary CBP levels. In contrast, *OCLN* and *ZO-1* expression showed neither significant overall treatment effects nor linear contrasts. Claudins are integral components of tight junctions that regulate the movement of paracellular ions and solutes [36]. Claudin-1 is generally regarded as a barrier-forming claudin that is involved in maintaining epithelial permeability [36]. Consistent with this function, Inai et al. [37] reported that overexpression of *CLDN1* in MDCK epithelial cells

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markedly increased TEER and reduced paracellular flux. Thus, the linear increase in *CLDN1* expression observed in the present study was consistent with the greater TEER observed with 0.10% CBP. However, because jejunal TEER and *CLDN1* mRNA expression were measured in different birds from the same replicate cages, the consistency between these findings should be interpreted with caution. Moreover, because *CLDN1* was assessed only at the mRNA level, these findings do not indicate that an increase in *CLDN1* expression was responsible for the increase in TEER. The absence of significant changes in *OCLN* and *ZO-1* expression did not necessarily conflict with the observed increase in TEER. Tight junction barrier properties depend on the composition and organization of junction proteins, and are regulated through processes such as protein trafficking and localization [36]. Accordingly, the present findings suggest that the increase in TEER was accompanied by a gene-specific linear response in *CLDN1* expression rather than by coordinated changes in the expression of all measured tight junction-related genes. However, the significant linear contrast observed for *CLDN1* expression should be interpreted cautiously, considering the multiple outcomes and statistical contrasts evaluated in the present study. Further studies are needed to identify the upstream mechanisms underlying these changes.

Several limitations should be considered when interpreting the present findings. First, only two low CBP inclusion levels (0.05 and 0.10%) and a single batch of CBP were evaluated, limiting the assessment of responses at higher inclusion levels and potential batch-to-batch variability. Second, the birds were not subjected to an experimental challenge, and TEER was the only direct functional measurement of jejunal epithelial barrier properties. Therefore, whether the observed TEER response would translate into meaningful intestinal or performance-related benefits under challenging conditions remains uncertain. Finally, only male birds were used for postmortem and intestinal analyses, limiting the generalizability of these findings to female broiler chickens.

CONCLUSION

Dietary supplementation with CBP at levels up to 0.10% did not significantly affect growth performance, meat quality, jejunal antioxidant capacity, jejunal morphology, or other physiological and welfare-related variables in broiler chickens. However, supplementation with 0.10% CBP increases jejunal TEER, indicating greater epithelial electrical resistance. In addition, *CLDN1* expression showed a significant linear response to increased dietary CBP levels. These findings indicate that the responses to CBP supplementation were observed in parameters related to intestinal barrier function rather than broadly across the measured outcomes. Thus, CBP may have potential as a feed material for modulating intestinal barrier function. However, the bioactive

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components and the mechanisms underlying these effects remain unclear. Further studies characterizing the bioactive composition of CBP and evaluating tight junction proteins and related barrier-regulatory mechanisms are required to clarify the intestinal barrier response to CBP supplementation in broiler chickens.

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458 **Table 1.** Composition and nutrient levels of experimental diets (as-fed basis)

Ingredient (%)	Starter (1 - 7 d)	Grower (8 - 21 d)	Finisher (22 - 35 d)
Corn	45.18	49.89	54.47
Wheat	8.00	10.00	8.00
Soybean meal (45% CP)	35.58	27.11	25.69
Tankage meal	5.00	6.00	4.63
Animal fat	3.12	4.41	4.66
MDCP	0.43	0.15	0.15
Limestone	1.21	1.22	1.25
Salt	0.15	0.15	0.15
55% L-Lys H_2SO_4	0.47	0.38	0.39
99% DL-Met	0.38	0.30	0.25
98% L-Thr	0.10	0.05	0.05
50% choline	0.18	0.14	0.11
$NaHCO_3$	0.10	0.10	0.10
Vitamin premix[1)]	0.05	0.05	0.05
Mineral premix[2)]	0.05	0.05	0.05
Total	100.00	100.00	100.00
Calculated energy and nutrient content			
AME_n (kcal/kg)	3,020.00	3,060.00	3,100.00
CP (%)	23.00	21.50	19.50
Digestible Lys (%)	1.36	1.22	1.11
Digestible Met + Cys (%)	1.03	0.95	0.89
Digestible Thr (%)	0.91	0.81	0.74
Digestible Trp (%)	0.22	0.20	0.18
Total calcium (%)	0.95	0.77	0.67

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Available phosphorus (%)	0.50	0.42	0.38

[1)]Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 μg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of pantothenic acid.

[2)]Provided per kg of diet: 37.5 mg of zinc (as $ZnSO_4$), 37.5 mg of manganese (as MnO_2), 37.5 mg of iron (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of copper (as $CuSO_4 \cdot 5H_2O$), 0.83 mg of iodine (as KI), and 0.23 mg of selenium (as $Na_2SeO_3 \cdot 5H_2O$).

CP, crude protein; MDCP, monodicalcium phosphate; AME_n, nitrogen-corrected apparent metabolizable energy.

ACCEPTED

Table 2. Chemical composition and antioxidant characteristics of cabbage by-product powder (CBP)[1)]

Items	CBP
Dry matter (%)	98.62 ± 0.16
Organic matter (%)	96.97 ± 0.04
Crude protein (%)	16.47 ± 0.42
Ether extract (%)	1.99 ± 0.01
Crude fiber (%)	14.05 ± 0.31
Gross energy (kcal/kg)	4,418.51 ± 7.25
Total phenolic content (mg GAE/g)	12.49 ± 0.22
Total flavonoid content (mg NE/g)	ND
DPPH radical scavenging activity (%)	50.88 ± 0.04

[1)]Except for dry matter content (as-fed basis) and DPPH radical scavenging activity (percentage inhibition), all values are expressed on a dry matter basis.

CBP, cabbage by-product powder; GAE, gallic acid equivalents; NE, naringin equivalents; ND, not detected; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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471 **Table 3.** The sequence of the primers used in quantitative real-time PCR

RNA target	Primer sequence	Accession no.
GAPDH	F: 5'-ATGGCATCCAAGGAGTGAGC-3'	NM_204305.2
	R: 5'-GGGAACAGAACTGGCCTCTC-3'	
OCLN	F: 5'-GTGGAGTCCAGTGATGAGCG-3'	NM_205128.1
	R: 5'-TGTCCATCTCAGCACAGAGC-3'	
CLDN1	F: 5'-GCTGACCTGTACTTGAGCTG-3'	NM_001013611.2
	R: 5'-TGGCACAGGGTTAATGCAAA-3'	
ZO-1	F: 5'-AGGTGAAGTGTTTCGGGTTG-3'	XM_015278975.1
	R: 5'-AGAAATCCGCTCGATCTCCT-3'	

472 *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *OCLN*, occludin; *CLDN1*, claudin-1; *ZO-1*, zonula
473 occludens-1.

ACCEPTED

474 **Table 4.** Effects of dietary supplementation of cabbage by-product powder (CBP) on growth performance in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
1 – 7 d							
Body weight (g)	208	202	204	3.2	0.464	0.359	0.409
Body weight gain (g)	163	158	159	3.2	0.463	0.357	0.409
Feed intake (g)	154	144	158	4.9	0.146	0.663	0.058
Feed conversion ratio (g/g)	0.95	0.91	0.99	0.028	0.159	0.259	0.116
8 – 21 d							
Body weight (g)	1,002	968	946	28.0	0.374	0.169	0.8714
Body weight gain (g)	795	766	742	26.1	0.385	0.173	0.943
Feed intake (g)	954	895	880	31.7	0.219	0.087	0.846
Feed conversion ratio (g/g)	1.21	1.17	1.19	0.041	0.973	0.847	0.899
22 – 35 d							
Body weight (g)	2,185	2,138	2,164	80.9	0.917	0.852	0.715

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Body weight gain (g)	1,184	1,144	1,219	58.4	0.671	0.677	0.435
Feed intake (g)	1,981	1,935	1,983	77.0	0.883	0.992	0.623
Feed conversion ratio (g/g)	1.69	1.71	1.64	0.069	0.752	0.587	0.607
1 – 35 d							
Body weight (g)	2,186	2,138	2,164	80.9	0.917	0.852	0.715
Body weight gain (g)	2,141	2,094	2,120	80.9	0.917	0.852	0.715
Feed intake (g)	3,203	3,041	3,093	98.1	0.507	0.439	0.388
Feed conversion ratio (g/g)	1.50	1.46	1.46	0.040	0.657	0.479	0.569

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder.

ACCEPTED

Table 5. Effects of dietary supplementation of cabbage by-product powder (CBP) on breast meat quality in broiler chickens[1)]

Items		Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
		0	0.05	0.10		T	L	Q
Breast yield (%)		24.3	23.8	25.4	0.59	0.195	0.210	0.186
pH	1 h	6.2	6.2	6.3	0.09	0.570	0.439	0.475
	24 h	5.7	5.6	5.7	0.03	0.403	0.656	0.208
Meat color	CIE L*	48.6	47.2	48.4	0.76	0.379	0.865	0.172
	CIE a*	2.4	2.6	2.4	0.30	0.823	0.947	0.541
	CIE b*	8.1	7.2	7.2	0.66	0.576	0.376	0.582
Water holding capacity (%)		72.0	73.2	71.0	1.37	0.516	0.614	0.306
TBARS (mg MDA/kg meat)		0.33	0.35	0.34	0.008	0.170	0.243	0.134

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

ACCEPTED

Table 6. Effects of dietary supplementation of cabbage by-product powder (CBP) on relative organ weight in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
Liver (%)	2.14	2.40	2.21	0.098	0.196	0.669	0.082
Spleen (%)	0.09	0.10	0.10	0.009	0.889	0.733	0.736
bursa of Fabricius (%)	0.36	0.36	0.38	0.040	0.881	0.662	0.813
Thymus (%)	0.24	0.27	0.27	0.034	0.748	0.476	0.807
Abdominal fat (%)	0.87	0.83	0.83	0.077	0.936	0.775	0.827

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder.

ACCEPTED

Table 7. Effects of dietary supplementation of cabbage by-product powder (CBP) on blood parameters in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
Total cholesterol (mg/dL)	140	140	143	6.8	0.919	0.716	0.860
Triglyceride (mg/dL)	24.6	23.4	25.3	2.02	0.809	0.806	0.552
HDL (mg/dL)	88.4	85.0	86.4	4.38	0.858	0.751	0.656
LDL (mg/dL)	33.6	35.0	36.1	3.82	0.893	0.639	0.976

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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Table 8. Effects of dietary supplementation of cabbage by-product powder (CBP) on liver parameters in broiler chickens[1)]

Items		Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
		0	0.05	0.10		T	L	Q
Liver color	CIE L*	22.0	22.9	21.2	0.61	0.158	0.329	0.096
	CIE a*	13.3	13.3	11.5	0.66	0.102	0.065	0.264
	CIE b*	2.8	2.6	2.1	0.32	0.278	0.124	0.706
Hemorrhagic score[3)]		0.06	0.00	0.09	0.048	0.348	0.509	0.213
Fatty liver score[4)]		1.06	1.06	1.03	0.049	0.993	0.921	0.954

[1)]Data are means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

[3)]Liver hemorrhage score: 0 = normal liver; 5 = extensive and severe hemorrhage [18].

[4)]Fatty liver score: 1 = normal liver; 5 = pale yellowish-red appearance [19].

CBP, cabbage by-product powder.

ACCEPTED

Table 9. Effects of dietary supplementation of cabbage by-product powder (CBP) on welfare-related indicators in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
Gait score	0.21	0.16	0.10	0.094	0.509	0.457	0.406
Footpad dermatitis	0.07	0.07	0.11	0.079	0.883	0.685	0.814
Hock burn	0.31	0.11	0.63	0.206	0.403	0.846	0.201
Feather cleanliness	0.10	0.04	0.11	0.036	0.417	0.752	0.219

[1)]Data are means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder.

ACCEPTED

Table 10. Effects of dietary supplementation of cabbage by-product powder (CBP) on jejunal antioxidant capacity in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
MDA (nmol/mg protein)	0.07	0.07	0.07	0.003	0.746	0.703	0.513
TAC (nmol trolox/mg protein)	81.69	90.58	86.63	4.430	0.383	0.440	0.252

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder; MDA, malondialdehyde; TAC, total antioxidant capacity.

ACCEPTED

Table 11. Effects of dietary supplementation of cabbage by-product powder (CBP) on jejunal morphology in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
Villus height (μm)	1,050	1,006	1,065	35.1	0.484	0.764	0.249
Villus width (μm)	144	148	152	4.1	0.393	0.178	0.973
Crypt depth (μm)	158	162	164	8.1	0.836	0.564	0.900
Villus height to crypt depth ratio	6.76	6.37	6.59	0.210	0.432	0.574	0.247

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

CBP, cabbage by-product powder.

ACCEPTED

Table 12. Effects of dietary supplementation of cabbage by-product powder (CBP) on jejunal permeability and tight junction-related gene expression in broiler chickens[1)]

Items	Inclusion levels of CBP in diets (%)			SEM	*p*-value[2)]		
	0	0.05	0.10		T	L	Q
TEER ($\Omega \cdot cm^2$)	153[b]	148[b]	172[a]	3.9	0.001	0.004	0.010
OCLN	1.00	1.03	1.02	0.092	0.977	0.873	0.888
CLDN1	1.00	1.16	1.28	0.086	0.111	0.041	0.812
ZO-1	1.00	1.08	1.12	0.096	0.668	0.388	0.845

[1)]Data are least squares means of 7 observations per treatment.

[2)]T, overall effects of treatments; L, linear effects of increasing inclusion levels of cabbage by-product powder in diets; Q, quadratic effects of increasing inclusion levels of cabbage by-product powder in diets.

[a,b]Means within a variable with no common superscript differ significantly ($p < 0.05$).

CBP, cabbage by-product powder; TEER, transepithelial electrical resistance; *OCLN*, occludin; *CLDN1*, claudin-1; *ZO-1*, zonula occludens-1.

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