

ISSN: (Print) (Online) Journal homepage: [www.tandfonline.com/journals/taar20](http://www.tandfonline.com/journals/taar20)

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To cite this article: Abdul Hafeez, Shahid Iqbal, Shabana Naz, Ibrahim A. Alhidary, Samia Abdelrahman & Maria Selvaggi (2024) Comparative effect of exogenous protease and phytobiotics on growth performance, nutrient digestibility, ileal histology and bone strength in broilers, Journal of Applied Animal Research, 52:1, 2399933, DOI: [10.1080/09712119.2024.2399933](https://doi.org/10.1080/09712119.2024.2399933)

To link to this article: <https://doi.org/10.1080/09712119.2024.2399933>



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Published online: 09 Sep 2024.



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## Comparative effect of exogenous protease and phytobiotics on growth performance, nutrient digestibility, ileal histology and bone strength in broilers

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### ABSTRACT

A total of 540 d-old Cobb 500 broiler chickens were randomly allocated into five groups: a control group receiving only the basal die (control), and experimental groups supplemented with exogenous protease (30,000 IU/kg) and crushed seeds of *Bunium persicum*, *Coriandrum sativum*, and *Carum copticum* (10 mg/kg each) for a period of 35 days. The results demonstrated that growth performance parameters, including feed intake, weight gain, and feed conversion ratio, were significantly improved ( $P < 0.05$ ) in broilers supplemented with protease compared to those receiving the selected herbs. Additionally, the digestibility of crude protein and crude fat showed a significant increase ( $P < 0.05$ ) in broilers that received protease compared to the *Bunium persicum*. Tibia bone weight significantly improved ( $P = 0.01$ ) in birds supplemented with protease, *Coriandrum sativum* and *Carum copticum*. In addition, the robusticity index decreased ( $P < 0.05$ ) in protease supplemented group. In terms of ileal histological features increased ( $P < 0.05$ ) in broilers supplemented with protease compared to the control. In summary, the results indicate that protease supplementation yielded superior outcomes in terms of improving growth, ileal digestibility, bone quality, and villus dimensions in broilers compared to the herbal plant supplements used in this study.

### ARTICLE HISTORY

Received 27 May 2024  
Accepted 29 August 2024

### KEYWORDS

Broiler; protease; *Coriandrum sativum*; *Bunium persicum*; *Carum copticum*

### Introduction



In contemporary broiler production, the reliance on antibiotics is often deemed unavoidable owing to their positive impact on broiler health and productivity (Hafeez, Shah et al. 2020; Imran and Alsayeqh 2022; Gul and Alsayeqh 2022a, 2022b; Al-Hoshani et al. 2023; Hussain et al. 2023). However, the widespread acknowledgment of their adverse effects has prompted significant scrutiny, leading to stringent restrictions on their unbridled use in many developed countries globally (Abudabos et al. 2018; Abbas and Alkheraije 2023; Quratulain et al. 2023). Consequently, there is an ongoing quest for non-antibiotic alternatives, including herbs, probiotics, organic acids, and other compounds, to sustain broiler growth without imparting adverse effects on end consumers (Jabbar et al. 2021; Salman and Imran 2022; Hegazy et al. 2022; Rashid et al. 2023).

*Coriandrum sativum*, commonly known as coriander, stands as a well-recognized herb with a rich history of use in traditional medicine for addressing various ailments. Extensive research has shed light on its multifaceted properties, including antioxidant, antifungal, hypocholesterolemic and hypolipidemic effects (Hosseinzadeh et al. 2014). The essential oil extracted from coriander contains notable alkaloids, such as pinede, linalool, geranyl acetate, terpinene, camphor, and geraniol (Burdock and Carabin 2009). Every part of the plant, from roots to leaves, fruits, and seeds, harbors a diverse array of chemical compounds, enhancing its versatility and applications

(Mandal and Mandal 2015). Noteworthy constituents include gallic acid, thymol, and bornyl acetate, each associated with specific health properties. Gallic acid is anticipated to exhibit anticancer properties, thymol is known for its anti-inflammatory effects, and bornyl acetate is expected to induce autonomic relaxation (Matsubara et al. 2011; Riella et al. 2012; Sun et al. 2016). Linalool, identified as a terpene alcohol present in coriander, has been characterized as the primary constituent responsible for certain therapeutic benefits (de Lucena et al. 2020).

*Bunium persicum*, commonly known as black cumin, is a distinguished herbal plant well known for its antimicrobial activities (Sekine et al. 2007). With a historical use for gastrointestinal issues such as dyspepsia and diarrhea, it has garnered attention for its antimicrobial and antioxidant properties (Tehseen et al. 2016). The seeds of black cumin are rich in a diverse array of phytochemicals, including aliphatic compounds, steroids, terpenes, flavonoids, phenolics, saponins, thymoquinone, tannins, resins, fatty acids, alkaloids, stigmasterol, campesterol, terpenoids, and esters. It is worth noting that wild or unripe *Bunium* seeds contain terpene hydrocarbons like  $\gamma$ -terpinene,  $p$ -cymene,  $\beta$ -pinene, and limonene, contributing to a lower quality of the spice (Sekine et al. 2007; Bansal et al. 2023).

*Carum copticum*, commonly known as Ajwain, is renowned for its effectiveness in addressing ailments of the digestive tract (Valiollahi et al. 2014). Studies have highlighted its

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**Table 1.** Composition of basal feed and chemical composition.

Ingredients	Starter phase	Finisher phase
Corn	55.3	55.50
Soybean meal	26.7	25.5
Canola meal	6.8	7.5
Sunflower meal	3.6	4.0
Vegetable oil	1.6	1.8
Molasses	2.5	1.0
Dialcium phosphate	1.5	2.0
Limestone	1.0	1.00
NaCl	0.2	1.00
NAHCO <sub>3</sub>	0.3	0.3
Vitamin mineral premix	0.5	0.5
Chemical composition		
ME, kcal/kg	3100	3000
Crude protein, %	22.0	21.50
Methionine, %	0.56	0.45
Lysine, %	1.45	1.22
Sulfur amino acids, %	0.95	0.85
Threonine, %	0.96	0.84
Calcium, %	1.0	0.91
Phosphorus, %	0.60	0.46

1Vitamin-mineral premix contains in the following per kg: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B2, 1600 mg; vitamin B6, 1000 mg; vitamin B12, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14000 mg.

antibacterial and antioxidant effects, suggesting potential benefits for improved feed utilization and enhanced growth in broilers (Falaki et al. 2016). These properties can be attributed to elevated levels of volatile oils, particularly Thymol, along with nicotinic acid (niacin), Paracymene, Terpinene, protein, fat,  $\beta$ -Pinene, saponins, glucosides, phenolic compounds (such as carvacrol), fiber, and minerals present in Ajwain seeds (Shafieezadeh et al. 2020).

Protease, an enzyme specialized in breaking down proteins, is widely employed as a supplement in broiler diets owing to its acknowledged capacity to enhance nutrient digestibility. Previous research has demonstrated notable benefits, including improved digestibility, enhanced bone strength and reduced abdominal fat in broilers with the addition of exogenous protease enzyme (Hafeez, Sohail et al. 2020; Jabbar et al. 2021). Importantly, there exists limited research that directly compares the effects of supplementing herbal plants and protease

enzyme. Consequently, the present study aims to evaluate and compare the impacts of protease enzyme and various herbal plants on key parameters such as growth performance, ileum histology, bone strength and nutrients digestibility in broilers.

## Materials and methods

### Broiler management

In this study, a total of 540 one-day-old broiler chickens from the Ross 308 breed were randomly assigned to five experimental treatments, each with five replicates. The control group received a basal diet (Table 1) comprising corn and soybean meal, while the remaining groups received a fine powder supplementation of *Coriandrum sativum*, *Carum copticum*, or *Bunium persicum* at a dosage of 10 mg/kg each. The active ingredients of each plant are provided in Table 2. A fourth experimental group received supplementation with a protease enzyme at a concentration of 30,000 IU/kg, sourced from *Bacillus subtilis*. The *Bacillus subtilis*-sourced protease was mixed into the feed ingredients, which were then thoroughly blended to ensure uniform distribution. The mixture was pelleted under controlled conditions to preserve enzyme activity and stored in a cool, dry place until feeding. This method ensures consistent enzyme effectiveness in the diet. This enzyme's primary function is to break down proteins and proteinaceous anti-nutrient factors found in plant materials, with an activity level of 600,000 units per gram. The supplementation commenced in the second week of the experiment and continued until the sixth week. The basal diet, comprising starter and finisher phases, was provided to the birds, with supplements mixed into the respective groups' basal diets. The feed was provided in pelleted form. Ingredients were carefully weighed and mixed to ensure uniform distribution. The mix was then processed into pellets using a standard pelleting machine to maintain feed quality and consistency. The broilers were raised on the floor with a stocking density of 10.5 birds/m<sup>2</sup>. Throughout the brooding phase, the room temperature was initially established at 34 ± 1.4°C and progressively adapted to the standard temperature of 23 ± 1.5°C. The overall duration of the experiment spanned 42 days.

### Growth performance

Weekly evaluations of feed intake, body weight, and feed conversion ratio (FCR) were carried out on a replicate basis.

### Apparent ileal digestibility (AID)

Chromium oxide, serving as an inert compound, was introduced at a rate of 5 g/kg, five days before the end of the experiment. The digesta samples were collected and immediately frozen at -20°C to prevent degradation. They were then thawed, spread evenly on trays, and dried in a forced-air oven at 60°C until a constant weight was achieved. This process ensured that the moisture was completely removed from the samples for accurate analysis. The AID of the broilers was assessed by randomly selecting 3 broilers per replicate, followed by decapitation with a sharp knife. After slaughtering,

**Table 2.** Proximate analysis and chemical composition of seeds of *Coriandrum sativum*, *Bunium persicum* and *Carum copticum* (per 100 g sample w/w).

Ingredients	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	<i>Carum copticum</i>
Crude fat, g	8.85	10.42	19.3
Crude protein, g	14.16	13.55	16.3
Crude fiber, g	36.58	21.53	12.8
Vitamins			
Vitamin C, mg	26	15	18
Vitamin E, mg	2.5	1.9	2.3
Vitamin A, $\mu$ g	331	233	184
Vitamin B complex, mg	1.9	1.6	1.7
Vitamin K, $\mu$ g	315	214	142
Minerals			
Calcium, mg	64	25	1.48
Iron, mg	1.35	2.6	13.2
Magnesium, mg	0.55	0.8	1.15
Phosphorous, mg	46	13	0.42
Potassium, mg	532	125	312
Sodium, mg	46	33	16
Zinc, mg	0.5	1.3	0.99

**Table 3.** Comparative effect of phytobiotics and protease on feed intake in broiler

Weeks	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	P value
1	132	129	119	127	140	2.39	0.15
2	286	285	282	287	290	2.66	0.95
3	527	505	503	495	515	4.91	0.11
<b>Starter</b>	944 <sup>a</sup>	919 <sup>ab</sup>	903 <sup>b</sup>	908 <sup>b</sup>	944 <sup>a</sup>	7.48	0.02
4	792	790	770	743	780	6.24	0.11
5	849	838	831	845	856	6.83	0.76
6	929	925	907 <sup>b</sup>	916	941	5.91	0.51
<b>Finisher</b>	2570 <sup>a</sup>	2553 <sup>ab</sup>	2508 <sup>b</sup>	2504 <sup>b</sup>	2577 <sup>a</sup>	14.5	0.04

Means having different superscripts in the same row shows significant difference ( $P < 0.05$ ).

**Table 4.** Comparative effect of phytobiotics and protease on weight gain in broiler.

Weeks	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	P value
1	115 <sup>b</sup>	112 <sup>b</sup>	114 <sup>b</sup>	103 <sup>c</sup>	124 <sup>a</sup>	2.35	<0.01
2	246 <sup>b</sup>	236 <sup>c</sup>	244 <sup>b</sup>	233 <sup>c</sup>	251 <sup>a</sup>	2.91	<0.01
3	321 <sup>ab</sup>	326 <sup>b</sup>	335 <sup>a</sup>	312 <sup>b</sup>	343 <sup>a</sup>	4.19	<0.01
<b>Starter</b>	682 <sup>b</sup>	674 <sup>b</sup>	694 <sup>b</sup>	648 <sup>c</sup>	718 <sup>a</sup>	8.25	<0.01
4	385 <sup>b</sup>	388 <sup>b</sup>	396 <sup>b</sup>	374 <sup>c</sup>	406 <sup>a</sup>	4.06	<0.01
5	342 <sup>b</sup>	333 <sup>b</sup>	345 <sup>b</sup>	321 <sup>c</sup>	359 <sup>a</sup>	5.19	<0.01
6	359 <sup>b</sup>	354 <sup>b</sup>	358 <sup>b</sup>	343 <sup>c</sup>	371 <sup>a</sup>	3.20	<0.01
<b>Finisher</b>	1085 <sup>b</sup>	1075 <sup>b</sup>	1098 <sup>b</sup>	1038 <sup>c</sup>	1135 <sup>a</sup>	11.3	<0.01

Means having different superscripts in the same row shows significant difference ( $P < 0.05$ ).

the skin was removed, and the abdomen was opened to collect ileal digesta. The dried digesta samples were then analyzed for AID (Hafeez et al. 2021) using the following formula:

Apparent ileal Digestibility

$$= 100 - \frac{\text{conc. of marker in feed}}{\text{conc. of marker in digesta}} \times \frac{\text{conc. of nutrient in digesta}}{\text{conc. of nutrient in feed}} \times 100$$

The analysis of phosphorus and calcium in the samples was performed using a spectrophotometer and atomic absorption, respectively, as outlined by Hafeez et al. (2021). In summary, 1 g of ileal digesta was combined with 10 ml nitric acid and 5 mL of perchloric acid for digestion, followed by heating until the fumes dissipated, and then allowed to cool. Approximately 50 ml of deionized water was added to the solution as the final product before evaluation.

### Ileal Histomorphology

In each replicate, three randomly selected birds were chosen randomly. After slaughtering, the abdomen was opened to remove ileum. A section of about 2.0 cm<sup>2</sup> was removed from the ileum, the cut piece was preserved in 10% formalin, and then embedded in paraffin. Staining with hematoxylin and

eosin was applied after slides were prepared. These slides were evaluated at 10× magnification under a microscope (Olympus, NV, Aartselaar, Belgium) for villus dimensions (Ahmad et al. 2024).

### Bone quality

Three birds were randomly selected from each replicate. The right tibia bones were separated from the already slaughtered birds, boiled at 100°C for 8 minutes, and then air-dried. Bone weight was measured using a digital balance. Other parameters were determined using the following formulas (Hafeez, Shah et al. 2020)

$$\text{Weight/length index} = \text{Weight (mg)} / \text{Length (mm)}$$

$$\text{Robusticity index} = \text{Bone length (mm)} / \text{Cube root of bone weight (mg)}$$

$$\text{Tibiotarsal index} = \text{Diaphysis diameter} - \text{Medullary canal diameter} / \text{Diaphysis diameter} \times 100$$

### Data analysis

Various parameters were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure (SPSS Inc., Chicago, IL). Treatment means were then compared using Tukey's test as a post-hoc test at a significance level of  $P \leq 0.05$ .

**Table 5.** Comparative of phytobiotics and protease on feed conversion ratio in broiler.

Weeks	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	P value
1	1.15	1.15	1.12	1.15	1.13	0.01	0.26
2	1.16	1.21	1.18	1.21	1.15	0.01	0.35
3	1.64 <sup>a</sup>	1.55 <sup>b</sup>	1.49 <sup>b</sup>	1.61 <sup>a</sup>	1.51 <sup>b</sup>	0.02	<0.01
<b>Starter</b>	1.39 <sup>a</sup>	1.32 <sup>a</sup>	1.32 <sup>b</sup>	1.39 <sup>a</sup>	1.32 <sup>b</sup>	0.01	<0.01
4	2.06 <sup>a</sup>	2.04 <sup>a</sup>	1.88 <sup>b</sup>	2.06 <sup>a</sup>	1.93 <sup>b</sup>	0.03	<0.01
5	2.49	2.52	2.47	2.60	2.39	0.04	0.79
6	2.59	2.61	2.56	2.64	2.54	0.02	0.23
<b>Finisher</b>	2.37 <sup>a</sup>	2.38 <sup>a</sup>	2.29 <sup>b</sup>	2.42 <sup>a</sup>	2.27 <sup>b</sup>	0.02	0.23

Means having different superscripts in the same row shows a significant difference ( $P < 0.05$ ).

**Table 6.** Comparative effect of phytobiotics and protease on apparent ileal digestibility in broiler.

Items (%)	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	<i>P</i> value
Dry Matter	73.4	72.3	73.1	72.2	73.6	0.35	0.44
Ash	46.4	46.9	47.4	46.7	47.1	0.37	0.60
Crude Protein	65.5 <sup>b</sup>	65.9 <sup>b</sup>	66.3 <sup>b</sup>	60.4 <sup>c</sup>	71.4 <sup>a</sup>	0.85	0.01
Crude Fat	73.7 <sup>b</sup>	74.1 <sup>b</sup>	74.8 <sup>b</sup>	69.1 <sup>c</sup>	79.3 <sup>a</sup>	0.80	0.02
Nitrogen Free Extract	81.2	82.5	82.1	79.0	84.8	0.50	0.09
Calcium	25.8	25.2	27.4	25.6	26.7	0.33	0.11
Phosphorus	24.9	25.3	25.4	24.9	25.5	0.27	0.68

Means having different superscripts in the same row shows significant difference ( $P < 0.05$ ).

## Results

The effects of phytobiotics and protease on feed intake in broilers are presented in Table 3. No significant changes were observed in feed intake within the initial 3 weeks of the experiment among the groups. However, during the overall starter phase, feed intake was notably higher ( $P < 0.05$ ) in the protease, control, and *Carum copticum*-supplemented groups compared to the broilers supplemented with *Coriandrum sativum* and *Bunium persicum*. Similarly, on a weekly basis, no significant changes were observed during weeks 4, 5, and 6 of the experiment between the control and treatment groups. Moving to the finisher phase, the protease-supplemented group and the control group recorded significantly ( $P < 0.05$ ) higher feed intake compared to the *Coriandrum sativum* and *Bunium persicum*-supplemented broilers.

The comparative effects of phytobiotics and protease on weight in broilers are illustrated in Table 4. The results indicated a significant increase in weight gain in the protease-supplemented group during weeks 1 and 2. Additionally, during week 3, higher ( $P < 0.05$ ) weight gain was observed in the protease, the Control group, and the *Coriandrum sativum*-supplemented groups. In the starter phase, weight gain was significantly ( $P < 0.05$ ) higher in the protease-supplemented group compared to the other groups. During weeks 4, 5, and 6, significantly ( $P < 0.05$ ) higher weight gain was observed in the protease-supplemented group of broilers compared to all other treatments. In the finisher phase, weight gain was significantly ( $P < 0.05$ ) higher in protease-supplemented broilers and significantly lower in *Bunium persicum*-supplemented broilers.

The comparative effects of phytobiotics and protease on feed conversion ratio (FCR) in broilers are presented in Table 5. It was observed that FCR was decreased with protease enzyme supplementation, *Carum copticum*, and *Coriandrum sativum* compared to the control and *Carum copticum* supplemented groups during week 3, although no significant change was noted during weeks 1 and 2. Similarly, in the starter phase, FCR decreased with protease enzyme supplementation and *Coriandrum sativum* compared to the control. In the 4th week, the same trend persisted; however, no significant change was observed during weeks 5 and

6. During the finisher phase, FCR was decreased ( $P < 0.05$ ) with protease enzyme supplementation and *Coriandrum sativum* compared to the control, *Carum copticum*, and *Bunium persicum*-supplemented broilers.

The comparative effect of protease enzyme, *Coriandrum sativum*, *Carum copticum*, and *Bunium persicum* on AID is presented in Table 6. No significant differences were observed in ash and dry matter between the control and experimental groups. However, protease-supplemented broilers exhibited significantly ( $P < 0.01$ ) higher crude fat and crude protein digestibility compared to the control and phytobiotic-supplemented broilers.

The outcomes of protease enzyme, *Coriandrum sativum*, *Carum copticum*, and *Bunium persicum* on bone quality are outlined in Table 7. Bone weight showed an increase ( $P < 0.05$ ) in protease, *Coriandrum sativum* and *Carum copticum*-supplemented birds. The robusticity index was decreased ( $P < 0.05$ ) in the protease-supplemented birds. However, Tibio-tarsal Index and Bone length did not exhibit significant changes ( $P > 0.05$ ) between the treatments.

The impact of protease enzyme, *Coriandrum sativum*, *Carum copticum*, and *Bunium persicum* on villus histology is detailed in Table 8. Villus width and length significantly increased ( $P < 0.05$ ) in broilers supplemented with protease, *Carum copticum*, *Coriandrum sativum*, and *Bunium persicum*. However, other parameters, including lamina propria, crypt depth, and the villus length to crypt depth ratio, did not exhibit significant changes ( $P > 0.05$ ). Villus surface area was significantly higher ( $P < 0.05$ ) in protease-supplemented, *Coriandrum sativum*, and *Carum copticum*-supplemented broilers compared to the control and *Bunium persicum*-supplemented broilers.

## Discussion

The current study addresses a notable gap in existing research by undertaking a comparative analysis of the impacts of various herbal plants and a protease enzyme on the performance, digestibility, bone strength, and ileal histology in broilers. To the best of our knowledge, such comprehensive investigations are scarce in the literature. The findings of this study indicate

**Table 7.** Comparative effect of phytobiotics and protease on bone quality in broiler.

	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	<i>P</i> value
Bone Weight (g)	6.02 <sup>b</sup>	7.16 <sup>ab</sup>	7.34 <sup>ab</sup>	5.55 <sup>b</sup>	8.97 <sup>a</sup>	0.32	0.01
Bone Length (mm)	89.9	87.8	86.5	93.9	90.4	0.80	0.11
W/L index	255	242	257	254	246	1.66	0.06
Robusticity Index	4.95 <sup>a</sup>	4.92 <sup>a</sup>	4.55 <sup>ab</sup>	5.12 <sup>a</sup>	4.16 <sup>b</sup>	0.09	0.01
Tibio-tarsal Index	32.5	34.7	36.9	32.8	31.0	0.78	0.37

Means having different superscripts in the same row shows a significant difference ( $P < 0.05$ ).

**Table 8.** Comparative effect of phytobiotics and protease on ileum morphology in broiler.

	Control	<i>Carum copticum</i>	<i>Coriandrum sativum</i>	<i>Bunium persicum</i>	Protease enzyme	Pooled SEM	<i>P</i> value
Villus Length (µm)	1.13 <sup>b</sup>	1.48 <sup>a</sup>	1.36 <sup>a</sup>	1.29 <sup>a</sup>	1.36 <sup>a</sup>	0.06	0.02
Villus Width (µm)	0.10 <sup>b</sup>	0.13 <sup>ab</sup>	0.14 <sup>a</sup>	0.11 <sup>b</sup>	0.13 <sup>a</sup>	0.01	0.01
Crypt Depth (µm)	0.24	0.25	0.24	0.25	0.23	0.01	0.90
Lamina Propria (µm)	0.06	0.06	0.06	0.06	0.06	0.00	0.22
Villus Length:C. Depth	4.81	6.36	5.98	5.46	5.98	0.34	0.16
Villus Surface Area (µm <sup>2</sup> )	0.37 <sup>b</sup>	0.61 <sup>a</sup>	0.60 <sup>a</sup>	0.46 <sup>b</sup>	0.59 <sup>a</sup>	0.04	0.01

Means having different superscripts in the same row show significant difference ( $P < 0.05$ ).

that birds supplemented with a protease enzyme exhibited higher feed intake during both the starter and finisher periods compared to those receiving herbal plants in their diet. Although there were no statistically significant differences in feed intake between the control group and the protease-supplemented broilers, there was a noticeable numerical increase in feed intake in the protease-treated groups. Conversely, broilers receiving herbal plant supplementation experienced a reduction in feed intake. Enzyme supplementation has garnered recognition for its nutritional, environmental, and economic benefits (Hafeez, Shah et al. 2020). Achieving optimal growth in young birds necessitates an increased intake of digestible protein. The complexity of soybean protein, its swift passage through the digestive system, and the deficiency of innate enzymes can significantly impact protein digestibility. Consequently, the inclusion of protease enzymes becomes imperative in the broiler diet, particularly during the critical starter phase. This addition is essential to address the challenges posed by the intricate nature of soybean protein, ensuring improved digestion and absorption for enhanced growth and overall well-being of young broilers (Jabbar et al. 2021).

In the present study, the introduction of exogenous protease supplementation demonstrated a correlation with increased feed consumption in birds. Comparable findings were also documented in prior reports (Hafeez, Shah et al. 2020; Hafeez, Sohail et al. 2020; Jabbar et al. 2021). The observed rise in feed intake among the birds could be attributed to their inclination to consume more feed, possibly to attain a balance in the required protein levels essential for sustaining growth. Conversely, in our study, a decrease in feed intake was noted in broilers supplemented with *Bunium persicum* and *Coriandrum sativum*. This outcome aligns with similar findings reported by Hafeez, Shah et al. (2020) and Khaligh et al. (2011), who observed a significant reduction in feed intake among birds receiving a diet supplemented with *Coriandrum sativum* at 10 g/kg compared to the control group. Additionally, Shroha et al. (2019) reported analogous results where *Coriandrum sativum* supplementation at 2, 4, and 6 g/kg led to a reduction in feed intake in broilers. Shuaib et al. documented an enhancement in feed intake among broilers supplemented with 0.5%, 1%, and 1.5% of *Bunium persicum*. The disparity in outcomes between their study and ours may stem from the lower dosage used in their research. The observed reduction in feed intake in our study could be linked to the presence of anti-nutritional factors, such as p-cymene and γ-terpinene in *Bunium persicum* (Foroumadi et al. 2002). These compounds might exert more pronounced negative effects, particularly at higher doses.

Divergent viewpoints exist among authors regarding the supplementation of feed additives of different plant origins on broiler feed intake. The conflicting results could be attributed to several factors, including the dosage and duration of supplementation, the presence of anti-nutritional factors, variations in broiler strains, differences in feed composition, and the influence of environmental factors (Hafeez, Shah et al. 2020).

In the current study, broilers supplemented with protease exhibited higher weight gain compared to both the control group and those receiving phytobiotic supplements. Exogenous enzymes, such as protease, demonstrate enhanced efficacy in nutrient utilization and growth by facilitating improved digestion (Dosković et al. 2013). Protease plays a crucial role in neutralizing anti-nutritional factors and enhancing the hydrolysis of proteins (Ghazi et al. 2002). Additionally, it elevates the availability and utilization of various amino acids. The literature corroborates our findings, with Freitas et al. (2011) reporting improved weight gain in broilers through the inclusion of protease at different concentrations (100, 200, 400, 800, and 1600 ppm) in the feed. In a similar vein, the study conducted by Hafeez, Shah et al. (2020) found that, when compared to protease enzyme supplementation, *Bunium persicum* and *Coriandrum sativum* did not exhibit a significant difference. Notably, birds supplemented with *Bunium persicum* showed a reduction in weight gain compared to those supplemented with protease, aligning with our study's results. This suggests that, in comparison to certain plant-based supplements, protease supplementation may be more effective in promoting weight gain in broilers. The reduced weight gain observed in broilers supplemented with *Bunium persicum* aligns with the findings of Shuaib et al., who reported higher weight gain at 0.5% compared to 1% and 1.5% *Bunium persicum* supplementation in broilers. In the present study, a significant decrease in Feed Conversion Ratio (FCR) was observed in broilers supplemented with protease compared to both the control group and birds treated with phytogenic additives. The improvement in FCR can be attributed to the corresponding values of feed intake and body weight, as FCR in broilers is inherently dependent on these factors. Therefore, the enhanced FCR observed in protease-supplemented broilers indicates improved efficiency in feed utilization and conversion to body weight.

Difference reports give different opinions in relation to the effects of the supplementation of *Coriandrum sativum*, *Carum copticum*, *Bunium persicum* and exogenous protease enzyme in broiler (Ghazi et al. 2002; Gracia et al. 2003; Onderci et al. 2006; Jang et al. 2007; Khaligh et al. 2011; Khan et al. 2012; Dosković et al. 2013; Shroha et al. 2019). The differences in results

have been linked to the feed composition, anti-nutritional factors, broiler strain, dose and duration of supplementations and environmental stressors (Khan et al. 2012). In the current study, the results obtained on the apparent nutrient digestibility in broilers in response to protease and herbs supplementation were not consistent. Compared to the control and herbs-supplemented broilers, crude protein and fat were significantly higher in protease enzyme-supplemented group. Previous studies have also reported better digestibility of nutrients in broilers in response to protease compared to herbal supplementations (Hafeez, Shah et al. 2020, Hafeez, Sohail et al. 2020, 2021). Due to the presence of several important compounds such as myrcene, limonene, p-cymene, b-pinene, and  $\gamma$ -terpinene, *Carum copticum* has been reported for many medicinal properties (Hassanzad Azar et al. 2018). *Carum copticum* seeds are potent medicine in digestive disorders and liver diseases (Ishikawa et al. 2001). Moreover, *Carum copticum* has been associated with antioxidant, antispasmodic, antimicrobial, and antifungal properties (Zarshenas et al. 2013). Similarly, *Bunium persicum* contains important compounds such as beta-pinene, cuminaldehyde, p-Mentha-1 and 4-dien 7-al, and gamma-terpinene. Previous studies have reported that a combination of *Bunium persicum* and *Carum copticum* in different concentrations improves apparent nutrient digestibility in broilers (Hafeez, Shah et al. 2020). Shuaib et al. (2020) also reported enhanced crude protein and fat digestibility in broilers with 0.5% *Bunium persicum* supplementation compared to the control, while other indicators of nutrient digestibility remained unchanged. In this study, protease supplementation yielded superior results for digestibility indices compared to the other treatments. Similar findings have been previously reported in broilers supplemented with protease (Jabbar et al. 2021). The positive effects of protease are attributed to reduced anti-nutritional factors, modification of intestinal microflora, and enhanced protein digestibility (Jabbar et al. 2021). These results are similar to the findings of Hafeez, Shah et al. (2020), Hafeez, Sohail et al. (2020), Hafeez et al. (2021). It can be inferred that nutrient digestibility in broilers is affected by several factors such as the dose of the medicinal plants and the stage of growth.

In the present study, a significant increase in bone weight was observed in birds supplemented with *Coriandrum sativum*, *Carum copticum* and protease enzyme, as compared to the control group. Leg issues pose a significant economic concern in broiler production, particularly given their rapid growth, which results in weaker bones compared to local breeds. Limited research has explored the impact of enzyme and medicinal plant supplementation on bone strength. Contrary to our findings, Hafeez et al. (2021) did not observe a significant effect on bone weight when broilers were supplemented with a combination of *Carum copticum*, *Bunium persicum* and *Trigonella foenum-graecum*. Hafeez et al. (2021) also reported increased bone weight in broilers supplemented with protease enzyme and *Coriandrum sativum*. Alam et al. (2020) also reported improved bone quality parameters in Japanese quails fed with phytase enzymes. The notable increase in bone weight in protease-enzyme-supplemented birds in our study may be linked to their weight gain or attributed to enhanced bone mineralization.

Furthermore, the robusticity index was significantly decreased in the protease and *Coriandrum sativum* groups, indicating stronger bones. This contradicts the results of Hafeez et al. (2021), where the robusticity index was decreased significantly in protease and herbal-supplemented broilers compared to the control. The variations in bone quality observed across studies suggest that factors such as the dosage of medicinal plants and the growth stage of broilers may influence bone health.

In the current study, villus length was significantly higher in protease and herbs-supplemented broilers compared to the control. However, villus width and villus surface area were significantly higher in *Carum copticum*, *Coriandrum sativum* and protease enzyme-supplemented broilers. In the study by Hafeez et al. (2021), histological features of the intestines were notably improved in broilers supplemented with protease enzyme and *Coriandrum sativum*. Previously, Alam et al. (2020) reported enhanced villus dimensions in Japanese quails supplemented with phytase enzyme. Similar findings were reported by Hafeez, Sohail et al. (2020) in an experiment with broilers fed *Trigonella foenum-graecum*, *Carum copticum*, and *Bunium persicum*. Limited research reports exist on the impact of herb supplementation on the skeletal system in response to various feed additives in broilers. Therefore, conclusively pinpointing the exact mechanism through which bone parameters are altered in broilers is challenging. However, based on the weight gain data in the current study, it seems that improved villus dimensions are correlated with enhanced weight gain in these birds, particularly in their respective groups, notably in the case of protease and *Coriandrum sativum*-supplemented broilers.

## Conclusion

Based on the findings, it can be concluded that growth performance, nutrient digestibility, bone quality, and ileal histological parameters were superior in broilers supplemented with protease compared to those supplemented with phytobiotics.

## Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project (No. RSPD2024R833), King Saud University, Riyadh, Saudi Arabia.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Ethical Approval

The Committee on Animal Rights and Welfare, GC University Faisalabad, Pakistan approved this study (DZ/34/2023)

## Consent to Participate

All authors have agreed to submit the article to this journal.

## Consent to Publish

All authors are agreed to publish in this journal.

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