



Effect of Canola Meal with and without Enzyme Supplementation on Growth Performance, Carcass Characteristics, and Serum and Bone Calcium and Phosphorus in Broiler Chickens

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ABSTRACT

This study assess the effect of different levels of canola meal (CM) with and without enzyme supplementation on the growth performance, carcass characteristics, and biochemical blood analysis of broiler chickens. A total of 240-day-old broilers were randomly assigned to 8 treatment groups with 3 replicates of 10 birds per cage. The treatments consisted of different levels of CM, with and without the addition of enzymes: CM₀ and CM_{0E} (control); CM₁₀ and CM_{10E} (10% CM with and without enzymes); CM₁₈ and CM_{18E} (18% CM with and without enzymes); and CM₂₅ and CM_{25E} (25% CM with and without enzymes). There were no significant differences ($P>0.05$) in growth performance between the control group and the groups fed 10% CM, irrespective of the level of enzyme supplementation. However, the birds fed CM at 18% inclusion with enzyme exhibited significant improvement in performance ($P<0.05$) compared to those fed on diet with 18% and 25% CM without enzyme supplementation. The levels of calcium and phosphorus in the blood serum increased significantly with higher CM levels, which also increased the ash content of the tibia. Birds fed on a diet containing 25% CM with enzymes demonstrated increased bone mineralization; though, this did not contribute to optimal growth of the birds. No significant differences in carcass weight were observed between control, CM at 10% with and without enzyme and 18% with enzyme supplement. For feed formulation in broiler diets, it is suggested to consider using either 10% CM without enzyme supplementation or 18% CM with enzyme supplementation. These options are safe with positive effect on the performance, carcass characteristics, and blood biochemistry.

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Key words

Performance, Serum calcium and phosphorus, Carcass traits, Broiler chicks, Canola meal

INTRODUCTION

The poultry industry contributes significantly to global food production, poverty alleviation and self-employment, and has experienced significant growth in recent years. However, despite its many benefits, the industry faces several challenges, including the rising cost of feed. The nutritional value and cost of feed ingredients are critical factors in determining the profitability of animal production. However, a thorough evaluation of the impact of alternative ingredients on animal growth

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performance is essential to ensure their effectiveness. The incorporation of cost-effective protein and amino acid sources into the diet stands as the most viable approach to reducing feed expenses in animal production. At the same time, it is important to carefully consider the impact of feed ingredients on the growth performance of host animals to accurately assess the viability of alternative ingredients (Wickramasuriya *et al.*, 2015). Feeding costs account for approximately 70% of the total cost of poultry production (Banday *et al.*, 2023). The need to explore cheaper and more accessible indigenous feed resources has become increasingly urgent in recent years, particularly due to rising feed costs and frequent disease outbreaks (Banson *et al.*, 2015). The scarcity of locally produced soybean meal in sufficient quantities and at affordable rates has forced poultry feed producers to explore alternative and cost-effective feed ingredients that do not compromise animal performance (Disetlhe *et al.*, 2018).

Canola meal derived from oilseeds, is gaining recognition as a promising ingredient in poultry nutrition (Fang *et al.*, 2007; Yadav *et al.*, 2022). It boasts a well-balanced amino acid profile and contains essential minerals and vitamins, including choline, biotin, folic acid, niacin, riboflavin, and thiamin (Spragg and Mailer, 2007; Wickramasuriya *et al.*, 2015). However, challenges arise from its high fibre content and the presence of anti-nutritional factors such as glucosinolates, tannins, sinapine, and erucic acid (Bell, 1993; Vahedifar and Wu, 2022). These factors restrict its inclusion levels in animal feeds, potentially compromising its antimicrobial properties. It has been observed that higher inclusion levels (>25%) can lead to a decrease in growth performance, likely due to the presence of anti-nutritional factors. Careful optimization of the use of canola meal is therefore essential to exploit its potential and ensure optimal poultry performance (Min *et al.*, 2011; Ahmed *et al.*, 2015; Disetlhe *et al.*, 2018).

Canola meal utilization in poultry diets is limited by anti-nutrients and high fiber content. Enzyme supplementation shows promise in enhancing its efficacy (Manyeula *et al.*, 2019). Some studies report no significant impact on chicken performance with high canola meal inclusion (Disetlhe *et al.*, 2018), while others demonstrate improved digestibility, feed utilization, and meat quality with enzyme complexes (Simbaya *et al.*, 1996; Kocher *et al.*, 2001; Ahmed *et al.*, 2015). On the contrary, (Slominski and Campbell, 1990; Sarıççek *et al.*, 2005) CM quality was improved when supplemented diet with enzymes. Several studies have shown improved digestibility, feed conversion efficiency and meat quality in broilers when xylanase, amylase and protease enzyme complex is added to the broiler diet (Cowieson and Ravindran, 2008; Yuan *et al.*, 2008; Yaqoob *et al.*, 2022). To improve performance

and nutrient bioavailability, this study aims to evaluate the effects of canola meal levels with and without enzyme supplementation on broiler growth, carcass characteristics and blood analysis.

MATERIALS AND METHODS

The current study was conducted at the Poultry Research Institute, Jabba, Mansehra, Pakistan and approved by the Departmental Animal Rights and Welfare Committee, University of Agriculture, Peshawar, Pakistan. The commercially available enzymes, quantum blue and β -mannanase, were purchased from the local market, Lahore, Pakistan. The activity of β -mannanase is 800,000 u/kg and its dose rate is 250-500 gm/tonne, while the activity of quantum blue is 5000 FTU/g and the dose rate is 50-500 gm/tonne. The appropriate amount of these enzymes was included in the experimental diets.

Birds, housing and experimental design

The chicks were reared in an open sided house in pens with good ventilation. Sawdust was provided as bedding on the floor. All essential equipment, including feeders and drinkers, was provided to ensure standard management and environmental conditions. The trial was lasted for 28 days provided the experimental rations from 7 to 28 days of age. On day 7, 240 broiler chicks of approximately the same weight and appearance was randomly alienated into 8 treatments. Four experimental diets were formulated to meet the minimum requirement of chicks as recommended by the National Research Council (NRC, 1994). The double zero variety of canola meal was incorporated in these diets at inclusion level of 0, 10, 18 and 25% with and without enzymes supplementation of the recommended dose of Quantum blue and β -Manganese were combined and was added to diets in a 4×2 factorial arrangement. The experimental treatments were as follows: CM₀ and CM_{0E} (0% CM without and with enzyme supplementation), CM₁₀ and CM_{10E} (10% CM without and with enzyme supplementation), CM₁₈ and CM_{18E} (18% CM without and with enzyme supplementation), and CM₂₅ and CM_{25E} (25% CM without and with enzyme supplementation) (Table I). All diets were isocaloric and isonitrogenous. Each diet was randomly assigned to 3 replicates of 10 chicks each.

Performance traits and nutrient digestibility

The initial weight of the chicks and then the weekly average body weight (BW) were recorded. Feed conversion ratio (FCR) was calculated accordingly. Body weight gain (BWG), feed intake (FI) and FCR were determined on a weekly basis. Three birds per replicate were slaughtered and carcass and liver weights were recorded.

Table I. Nutrient composition and calculated analysis of experimental starter diets.

Ingredients (%)	Con- trol	10% CM	18% CM	25% CM
Corn	56.20	53.97	53.62	51.32
Soybean meal (48%)	32.13	22.46	12.25	6.53
Canola meal	0.00	10.00	18.00	25.00
Corn gluten meal (60)	2.10	3.55	4.50	4.70
Fish meal	2.90	2.75	4.75	5.00
Vegetable oil	2.49	3.19	3.30	4.00
Molasses	1.00	1.00	1.00	1.00
Ground limestone	1.29	1.21	1.04	0.97
Dicalcium phosphate	1.23	1.21	0.90	0.83
Iodized Salt	0.42	0.43	0.42	0.42
DL-mthionine	0.12	0.11	0.10	0.11
Premix ¹	0.12	0.12	0.12	0.12
Total	100	100	100	100
Calculated analysis				
CP (%)	23	23	23	23
ME (kcal/g)	3.07	3.07	3.07	3.07
Calcium (%)	1.00	1.00	1.00	1.00
Av. phosphorus (%)	0.45	0.45	0.45	0.45
Lysine (%)	1.26	1.22	1.22	1.23
Mthionine (%)	0.53	0.54	0.57	0.59
Meth. and cystein (%)	0.90	0.90	0.92	0.93

¹Supplied per kilogram of diet: vitamin A, 450 U; vitamin D3, 300 U; vitamin E, 25 mg; vitamin C, 11 mg; vitamin K3, 16 mg; vitamin B1 3 mg; vitamin B2 6 mg; vitamin B2, 16 mg; vitamin B6, 16 mg; vitamin B12, 0.2 mg; choline chloride 400 mg; Ca pantothenate, 45 mg; folic acid, 0.4 mg; Fe, 42 mg; Cu, 3.7 mg; I, 0.5 mg; Zn, 54 mg; Mn, 90 mg; Mg, 108 mg; Ca, 11 mg; Cl, 671 mg; K, 78 mg; Met, 45 mg; niacin, 25 mg.

Collection of blood samples

Before slaughtering the birds, 4 ml of blood was collected from the wing vein of each duplicate using a 5 ml syringe. To separate serum, the blood were centrifuged at 3000 rpm and serum was collected in a test tube that was labeled, sealed, and kept at 0°C. Serum characteristics were determined in National Veterinary Laboratory Islamabad, according to protocol described for calcium (Connerty and Briggs, 1966), and phosphorus (Gomori, 1942).

Tibia bone collection and CP digestibility

At the end of the experiment, three birds from each replicate were sacrificed and the left tibia was removed from each bird. Meat and blood stains were removed from these tibia bones and placed in polythene bags, labelled and stored in a freezer until analysis. For analysis,

tibia bones were air dried at 60 °C for 24 h. Tibia bones were then stored in a muffle furnace at 550 °C for 4 h for incineration. The crucible was then transferred to a desiccator for cooling and after 30 min, all samples were ground to powder and stored in labelled polythene bags.

For digestion, two grams of sample was taken into a beaker and 20 ml of nitric acid was added with a pipette and kept until it became colorless. Then 10 ml perchloric acid was added to each sample and kept in fume hood for digestion. Through a Watt man filter, the digested sample was separated and made up to 100 ml with distilled water. Finally, all these samples were analyzed according to AOAC procedures (official methods number 964.06 and 927.02, respectively; AOAC, 1995) for the determination of calcium and phosphorus. To determine nutrient digestibility, chromium oxide (Cr2O3) was added to the experimental diets at a level of 0.3% on day 25, chromium oxide was fed for the next 3 days. Feces were collected at 28 days of age and the dry matter content of the diets and feces samples was determined by oven drying at 105°C. Cr2O3 was determined by the method described by Dansky and Hill (1952).

Statistical analysis

The final data were recorded in Microsoft Excel worksheets for sorting and basic statistics. Data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2006). Duncan multiple range tests were used to separate means. A probability (P) value less than or equal to 0.05 was considered statistically significant.

RESULTS

Performance

The average BWG, FI and FCR of chicks fed different levels of CM during the first, second and third weeks of the experiment and the total body weight gain are shown in Table II. There was no significant difference ($P>0.05$) in BWG and FCR of chicks fed up to 10% CM compared to chicks fed CM0 diet. However, BWG and FCR were reduced ($P<0.05$) with a gradual increase of CM in the diets. There was no significant effect of the canola meal supplementation-based diet on the FI throughout the experiment.

Crude protein (CP) digestibility

The CP digestibility observed in broiler chicks when fed diets containing different levels of CM, with and without enzyme supplementation, is shown in Table III. CP digestibility was highest in chicks fed diets

Table II. Effect of different inclusion levels of canola meal with and without enzyme supplementation on the performance of broiler chicken

Parameters	Treatments							
	CM ₀	CM _{0E}	CM ₁₀	CM _{10E}	CM ₁₈	CM _{18E}	CM _{25E}	CM _{25E}
7-14 days								
BWG (g)	255.0 ^{bc} ±8.76	268.33 ^{ab} ±11.55	261.67 ^{ab} ±10.41	273.33 ^a ±7.64	231.33 ^d ±7.65	253.33 ^{bc} ±5.77	230.00 ^d ±9.01	240.50 ^{cd} ±8.67
FI (g)	421.73±15.65	456.42±26.79	426.50±36.87	438.83±24.21	430.13±26.76	443.53±16.63	445.67±21.52	429.08±21.25
FCR (g/g)	1.65 ^{cd} ±0.06	1.70 ^{cd} ±0.06	1.63 ^c ±0.08	1.61 ^c ±0.05	1.86 ^{ab} ±0.07	1.75 ^b ±0.09	1.94 ^a ±0.06	1.78 ^{bc} ±0.04
15-21 days								
BWG (g)	375.0 ^{abc} ±11.03	388.42 ^{ab} ±8.73	385.00 ^{ab} ±7.55	389.83 ^a ±6.25	362.33 ^{cd} ±10.40	373.07 ^{bc} ±8.53	354.70 ^d ±9.33	362.83 ^{cd} ±6.79
FI (g)	586.65 ^a ±11.91	571.04 ^{ab} ±32.96	598.50 ^a ±13.31	594.50 ^a ±11.30	560.33 ^{ab} ±10.02	592.11 ^a ±28.67	544.69 ^b ±13.77	569.25 ^{ab} ±20.97
FCR (g/g)	1.57 ^{ab} ±0.08	1.47 ^b ±0.06	1.56 ^{ab} ±0.07	1.53 ^{ab} ±0.01	1.55 ^{ab} ±0.05	1.59 ^a ±0.06	1.54 ^{ab} ±0.03	1.57 ^{ab} ±0.04
22-28 days								
BWG (g)	481.67 ^{bc} ±10.60	501.75 ^a ±6.74	470.35 ^{cd} ±9.04	499.56 ^a ±4.82	436.96 ^f ±10.76	492.30 ^{ab} ±9.47	448.46 ^{ef} ±7.71	459.17 ^{de} ±8.78
FI (g)	825.00 ^a ±8.89	815.67 ^a ±8.14	824.00 ^a ±5.29	823.67 ^a ±6.03	818.33 ^a ±12.58	814.33 ^{ab} ±5.13	802.33 ^{bc} ±2.52	800.33 ^c ±1.53
FCR (g/g)	1.7 ^c ±0.05b	1.62 ^d ±0.02	1.75 ^b ±0.05	1.65 ^{cd} ±0.03	1.87 ^a ±0.08	1.66 ^{cd} ±0.04	1.79 ^b ±0.04	1.74 ^b ±0.04
7-28 days								
BWG (g)	1111.6 ^b ±11.06	1158.50 ^a ±26.68	1133.52 ^{ab} ±22.82	1146.22 ^{ab} ±21.04	1030.63 ^c ±25.32	1118.70 ^{ab} ±14.12	1033.17 ^c ±25.87	1062.50 ^c ±22.22
FI (g)	1833.33±11.56	1843.13±58.48	1857.33±30.27	1848.67±21.83	1808.80±23.76	1849.97±47.75	1792.69±33.27	1798.67±39.21
FCR (g/g)	1.65 ^{cd} ±0.02	1.59 ^c ±0.02	1.64 ^d ±0.01	1.61 ^c ±0.02d	1.75 ^a ±0.03	1.65 ^{cd} ±0.05	1.73 ^{ab} ±0.03	1.69 ^{bc} ±0.03

Means with different superscripts along a row are different significantly ($p < 0.05$). BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; CM₀ = 0% Canola meal, CM_{0E} = 10% Canola meal with enzymes; CM₁₀ = 10% Canola meal, CM_{10E} = 10% Canola meal with enzymes, CM₁₈ = 18% Canola meal, CM_{18E} = 18% Canola meal with enzymes; CM₂₅ = 25% Canola meal, CM_{25E} = 25% Canola meal with enzymes.

Table III. Effect of different inclusions of canola meal with and without enzymes supplementation CP digestibility, blood serum Ca and P and Tibia Ca and P.

Parameters	Treatments							
	CM ₀	CM _{0E}	CM ₁₀	CM _{10E}	CM ₁₈	CM _{18E}	CM ₂₅	CM _{25E}
Digestibility (%)								
CP	69.75 ^{bc} ±0.38	71.52 ^a ±0.25	69.23 ^{cd} ±0.16	71.32 ^a ±0.33	68.90 ^d ±0.39	70.26 ^b ±0.71	66.09 ^f ±0.50	67.58 ^c ±0.54
Blood serum (mg/100ml)								
Ca	6.53 ^d ±0.50	8.77 ^b ±0.16	6.68 ^d ±0.35	8.32 ^b ±0.58	7.37 ^c ±0.26	8.87 ^b ±0.28	6.87 ^{cd} ±0.30	10.88 ^a ±0.19
P	3.48 ^{ef} ±0.24	5.15 ^{ab} ±0.66	3.13 ^f ±0.13	4.53 ^c ±0.25	3.82 ^{de} ±0.26	4.22 ^{cd} ±0.18	4.68 ^{bc} ±0.25	5.65 ^a ±0.22
Tibia (%)								
Ash	42.18 ^d ±0.23	43.22 ^c ±0.20	42.35 ^d ±0.17	43.88 ^b ±0.15	43.29 ^c ±0.13	44.14 ^b ±0.14	44.00 ^b ±0.12	44.53 ^a ±0.22
Ca	22.14 ^d ±0.27	23.98 ^a ±0.22	23.29 ^b ±0.27	23.79 ^a ±0.34	22.22 ^d ±0.24	23.29 ^b ±0.27	22.74 ^c ±0.30	23.02 ^b ±0.23
P	15.52 ^b ±0.30	16.60 ^a ±0.30	14.03 ^d ±0.32	14.87 ^{bc} ±0.42	14.58 ^{cd} ±0.65	14.96 ^{bc} ±0.56	15.16 ^{bc} ±0.38	16.62 ^a ±0.35

Means with different superscripts along a row are different significantly ($p < 0.05$). BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio. CM₀ = 0% Canola meal, CM_{0E} = 10% Canola meal with enzymes; CM₁₀ = 10% Canola meal, CM_{10E} = 10% Canola meal with enzymes, CM₁₈ = 18% Canola meal, CM_{18E} = 18% Canola meal with enzymes; CM₂₅ = 25% Canola meal, CM_{25E} = 25% Canola meal with enzymes.

containing CM_{0E} and CM_{10E} with and without enzyme supplementation. It gradually decreased in chicks fed diets containing 18% and more CM. The enzyme supplementation was pronounced ($P < 0.05$) at each level of CM inclusion in the diet compared to the diet at different levels without enzyme supplementation.

Blood serum Ca and P contents

The effect of feeding different levels of CM with or without enzyme supplementation was found to cause major variations ($P < 0.01$) in the blood serum Ca content (Table III). Significant interaction was found between the different levels of CM and enzyme supplementation on

the blood serum Ca content. The Ca content in the blood serum was lowest ($P < 0.05$) in chicks on diets containing up to 10% CM. It was increased significantly above 10% CM inclusion in the diets. Enzyme supplementation at each level of CM in the diet showed significant response in the blood serum Ca content of chicks. There was 34.3, 24.6, 20.4 and 58.45% more serum Ca content in chicks when enzymes were supplemented in diets containing 0, 10, 18 and 25% CM than chicks on the same diets but without enzyme supplementation, respectively. The magnitude of increase in the Ca content was more at higher than low level of CM inclusion in the diet. The broiler diet containing 25% CM with enzyme supplementation showed the highest ($P < 0.05$) serum Ca content.

The interaction between the supplementation of enzymes and the level of CM in the diets was found to have a significant effect on the P content of the blood serum. Serum P levels were similar in chicks fed diets containing up to 10% CM, but CM of 18% and more in the diet increased serum P levels in chicks ($P < 0.05$). Enzyme supplementation at any level of CM in the diet significantly increased serum P levels in chicks. There was 48 and 44.7% more serum P in chicks on the CM_{0E} and CM_{10E} diets than on the CM_0 and CM_{10} diets, respectively. Interestingly, a higher level of CM (25%) inclusion in the broiler diet caused a significant increase in blood serum P content and was accounted to be 34.5% more in chicks fed on CM_{25} than CM_0 diets. The increase in serum P between these diets was increased to 62.4% when the enzyme was supplemented (CM_{25E} vs. CM_0). However, this increase in serum P in chicks fed diets containing 25% CM, with and without enzyme supplementation, did not improve growth performance.

Carcass weight and liver weight

Carcass weight decreased significantly ($P > 0.05$) when chicks were fed 25% CM, as shown in Figure 1A. The liver weight of chicks fed 10% CM was lower than that of chicks fed no CM (CM_0), although the difference was not statistically significant (Fig. 1B). The liver weight of chicks fed 25% enzyme-supplemented CM was significantly higher than that of chicks fed 10% enzyme-supplemented CM ($P < 0.05$).

DISCUSSION

In the present research, enhanced BWG and FCR were observed in chickens fed up to 10% canola meal without enzyme supplementation, and up to 18% with enzyme supplementation. Although feeding canola meal showed decreased growth performance than control group, no difference was observed between 10 and 18% inclusion.

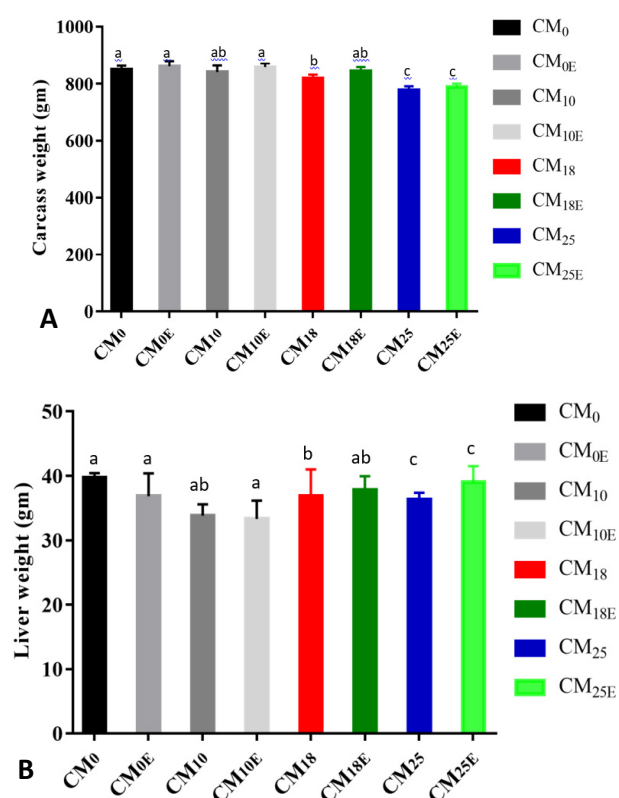


Fig. 1. The effect of canola meal on the carcass weight (A) and liver weight (B) of chickens. CM_0 =0% Canola meal, CM_{0E} =10% Canola meal with enzymes; CM_{10} =10% Canola meal, CM_{10E} =10% Canola meal with enzymes, CM_{18} =18% Canola meal, CM_{18E} =18% Canola meal with enzymes; CM_{25} =25% Canola meal, CM_{25E} =25% Canola meal with enzymes.

The present study in agreement with a previous experiment reporting that canola meal can only be included up to 10% for starter chicks and maximum 20% during a grower phase (Yadav *et al.*, 2022), and below 20% inclusion (Payvastagan *et al.*, 2012). Similarly, CM was added up to 16.7% in diets for broilers without affecting growth performance (Gopinger *et al.*, 2014). In our study, a decreased performance was observed by the inclusion level of 25% canola meal without enzymes supplementation, which is not in line with that observed in the previous study (Min *et al.*, 2011). It may be because higher levels of canola meal in the broiler diets make it less competitive with soybean meal due to low available energy (Slominski and Campbell, 1990) and increased fiber content especially non-starch polysaccharides (Francesch and Brufau, 2004; Buchanan *et al.*, 2007). Similarly, glucosinolates and other anti-nutritional factors may possibly reduce growth (Khajali and Slominski, 2012). Canola meal inclusion

although decreased body weight of birds, both 20 and 40% were able to maintain feed efficiency (Yadav *et al.*, 2022).

In current study, we found that canola meal can be added up to 18% to 25% with enzymes supplementation without any negative effect on the CP digestibility. Similar results were also obtained, when canola meal was added up to 20% in broiler chicken diet without any negative effect on the CP digestibility (Gopinger *et al.*, 2014). It has been shown that high amount of fiber in the meal (Bell, 1993) and high temperature during the processing of canola seed may negatively affect protein nutrient digestibility (Slominski, 1997), that ultimately affect the overall nutritional value of canola meal. Further, dietary fiber reduces nutrient digestibility due to its physiochemical properties, leading to a more rapid rate of passage that limits the amount of time available for nutrient breakdown (Thacker and Petri, 2011). However, increase in dietary fiber can be the explanation for the reduction in nutrient digestibility in the present trial as fiber crude levels increased as the amount of canola meal in the diet increased. The of total dietary fiber values for canola meal are higher than those of SBM due to a much higher content of lignin with associated polyphenols (Gopinger *et al.*, 2014).

The diets containing 25% CM with and without enzyme supplementation failed to improve performance in broiler chicks indicated that increasing level of CM in the diet has adverse effect on the performance of broiler chicks. At 10% CM in the diet, the carcass weight in chicks with and without enzymes supplemented were similar indicating that the anti-nutrients at this level of CM in the diet were sufficiently below the marginal level to cause problem. When the level of CM in the diet was increased the performance started to decrease. The negative effect of 18% CM inclusion was diminished when enriched with enzymes. The improved carcass weight of chicks as a result of enzyme supplementation might be due to the increased CP digestibility (Selle *et al.*, 2006).

The P retention in tibia ash was decreased up to 18% canola meal inclusion in the diet. However, when 25% canola meal was included, the P retention was similar to the control, revealed that higher level of canola meal in term of P retention respond differently than low CM meal diet. The interaction between enzyme supplementation and CM levels in the diets was found to have significant influence on the blood serum Ca and P contents. Enzyme supplementation at each level of CM in the diet showed significant response in the blood serum Ca and P contents. The Ca content in the blood serum was lowest ($P < 0.05$) in chicks on diets containing up to 10% CM. It was increased significantly above 10% CM inclusion in the diets. The magnitude of increase in the Ca and P contents was more at higher than low level of CM inclusion in the diet.

Interestingly, higher level of CM (25%) inclusion in the broiler diet caused significant increase in the blood serum P content and was accounted to be 34.5% more in chicks on CM₂₅ than CM₀ diets. The increase in serum P content between these diets were raised to 62.4% when enzyme was supplemented (CM_{25E} vs. CM₀). However, this increase of serum Ca and P contents in chicks on diet containing 25% CM with and without enzyme supplementation did not improved growth performance.

Feeding different levels of CM with and without enzyme supplementation caused significant differences in the bone quality of chicks. There was gradual increase in the tibia ash and its Ca and P contents with the increasing level of canola meal in the diet. Enzyme supplementation at each level of CM showed increase bone mineralization in chicks. The effect in tibia P was more pronounced in chicks on diet having 25% canola meal with enzymes supplemented diet. The tibia Ca content in chicks on diet CM₂₅ was better ($P < 0.05$) than chicks on diet CM₀. It seems that the enzymes were more active at higher CM inclusion in liberating more P and Ca from the phytate P complex (Sebastian *et al.*, 1996). However, this increase in the form of tibia ash and its Ca and P contents did not support optimum growth performance indicated that increased bone mineralization was not good indicator of increased growth performance in chicks reared on diets containing 25% CM with and without enzyme supplementation. It is recommended to further probe into the effect of enzymes on the minerals retention with emphasis that why the increased bone mineralization at 25 % CM did not support optimum growth performance.

In conclusion, the inclusion of canola meal up to 10% without enzyme and 18% with enzyme supplementation in the basal diet was found to be safe without affecting the performance of broiler chicks. Feeding 18% CM without enzyme in the diet reduced growth performance and feed efficiency of broiler chicks.

DECLARATIONS

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IRB approval

This study was approved by the animal welfare and care committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools was considered to minimize the pain and discomfort of birds during the conduction of this experiment.

Ethical statement

The study was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences (FAHVS), The University of Agriculture Peshawar, before the conduction of this experiment.

Generative AI and AI-assisted technology statement

The authors have declared that no generative AI or AI-assisted technologies were used to create this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest

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