

Impact of Rapeseed Meal with Protease Supplementation on Growth Performance, Nutrient Digestibility, and Carcass Characteristics in Broiler Chickens

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ABSTRACT

A total of nine hundred (900) healthy Ross[®] 308 one-day-old chicks weighing $42 \text{ g} \pm 3 \text{ g}$ were allocated into nine different dietary treatments having 4 replicates in each, containing 25 birds each replicate. Birds were treated with 9 different iso-nitrogenous and iso-caloric diets using three different inclusion levels of rapeseed meal (RSM) at 0%, 6% and 12% supplemented with three different dosages of protease enzyme i.e., no protease (0 gm/ton of feed), single dose (300 gm/ton of feed) protease, and double dose (600 gm/ton of feed). Results indicated that RSM inclusion had no significant ($P > 0.05$) impact on body weight gain (BWG) during the early phase (1-7 days), but higher RSM levels significantly ($P < 0.05$) reduced BWG and increased feed conversion ratio (FCR) in subsequent phases. Broilers fed 0% RSM showed significant ($P < 0.05$) increase in feed intake (FI). Protease supplementation at double dosage with 6% RSM improved BWG, FI. However, FCR improved with single dosage of protease both in grower and finisher phases ($P < 0.05$). Digestibility of dry matter (DM) and crude protein (CP) improved in birds fed RSM at 6% and 12% with single dosage of protease during grower and finisher phases ($P < 0.05$). However, no significant effect noticed with 0% RSM. Carcass and thigh weight showed significant increase ($P < 0.05$) in broilers fed 0% and 6% RSM with single dose protease. Interestingly, drumstick and liver weights across the treatment groups were non-significant ($P > 0.05$), however, heart weight showed a significant ($P < 0.05$) increase in 12% RSM group having no protease supplementation. In conclusion, it is suggested that RSM inclusion at 6% with protease supplementation in broiler diet improves growth performance and nutrient digestibility and carcass characteristics in broiler chicken.

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Authors' Contribution

QS: Conceptualization, methodology, investigation, data curation, writing original draft, formal analysis.

SAP: Supervision, formal analysis, validation, resources. ABS: Writing review and editing, formal analysis, software. WA: Writing review and editing, software.

Key words

Broiler, Carcass characteristics, Nutrient digestibility, Poultry, Protease, Rapeseed meal

INTRODUCTION

In poultry farming, the major expense is poultry feed which contributes approximately 70% of total cost (Thirumalaisamy *et al.*, 2016). Poultry feed is formulated according to bird's nutritional requirement including high quality nutrients like energy, protein, amino acids, calcium, and phosphorus at all the stages of rearing for better growth and productivity (Applegate and Angel, 2014).

Protein is a major component in poultry feed which has a core impact on feed costing (Thirumalaisamy *et al.*, 2016). Soybean meal (SBM) is the main source of protein used in poultry feed and with sustained rise in the price of SBM demanding pressure has been diverting towards the least cost protein source for advance poultry production (Karkelanov *et al.*, 2021; Whiting *et al.*, 2019). Major part of protein for poultry feed is derived from plants sources however in plant-based feed ingredients contain anti-nutritional factors (ANF) which affect the feed utilization (Erdaw and Beyene, 2018).

Rapeseed meal (RSM) is a by-product of rapeseed produced during oil recovery process which is used as alternate and attractive protein source for poultry feed (Kasprzak *et al.*, 2016; Olukosi *et al.*, 2017). Due to high protein content and comparatively low-cost RSM widely used as protein source in poultry feed. With less sustainability in the price of SBM managing low-cost alternative protein source might be best option in broiler

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feed composition (Cowieson and Bedford, 2009). Studies have shown that protein content of rapeseed meal is 14-16% less digestible compared to SBM protein or casein protein in standard diet (Adedokun *et al.*, 2008). RSM has crude protein (CP) content of around 35-40% and is rich in essential amino acids, especially lysine, methionine and cystine (Pastuszewska *et al.*, 2003). One of the major drawbacks of RSM is its ANF such as glucosinolates and phytates, as well as a high content of fiber and lignin from its seed hulls which have negative impact on protein utilization and digestibility that ultimately prevents the acceptance of RSM in feed industry (Kasprowicz-Potocka *et al.*, 2024; Mansour *et al.*, 1993).

Protein digestibility has a major impact on bird performance which directly affects the selection of RSM in poultry industry because of its low protein digestibility as compared to soybean meal (Fuller and Tomé, 2005). Proteases break down protein molecules into peptides and amino acids that further absorb within the small intestine particularly in ileum. Endogenous proteases released in the gastrointestinal tract are responsible for digestion of protein in birds. However, there is limited secretion of endogenous protease to digest a high protein diet (Recoules *et al.*, 2017). Exogenous microbial proteases are increasingly being added in broiler feeds to enhance the solubility and digestion of dietary protein and amino acids which provide financial benefits (Adeola and Cowieson, 2011; Caine *et al.*, 1998). Several studies have shown the effects of exogenous proteases on protein digestibility (Freitas *et al.*, 2011; Liu *et al.*, 2013; Stefanello *et al.*, 2016), performance (Angel *et al.*, 2011; Ghazi *et al.*, 2002; McCafferty *et al.*, 2022), immunity (Cowieson *et al.*, 2016) and intestinal health of birds (Cowieson *et al.*, 2018; Xu *et al.*, 2017).

We hypothesized that the effect of exogenous protease may improve the protein utilization of RSM which would directly impact on performance, nutrient digestibility, and carcass characteristics of birds. As for our knowledge, no studies have been conducted to study the effect of protease supplementation on various levels of RSM. Therefore, current study has been performed to assess the effect of protease on various levels of RSM on broilers birds of different age.

MATERIALS AND METHODS

Experimental design, housing and feeding management

Current study was conducted at Poultry Experimental Station at Sindh Agriculture University, Tandojam, Pakistan. The house preparation was conducted a day before chick arrival followed by dry washing and wet washing with effective disinfectant and formalin spray

throughout the shed. Rice hulls were used as bedding material. The temperature of the house kept at 95°F and kept on decreasing each week till it reached 75°F. A total of 900 feed efficient, fast growing and versatile one-day-old healthy broiler chicks of Ross® 308 (M/s Aviagen®, USA), weighing 42 g ± 3 g, randomly assigned to nine different dietary groups, with each group consisting of 4 replicates. Each replicate comprised of 25 chicks and housed in an individual pen. To conduct the experiment, nine iso-nitrogenous and iso-caloric diets were formulated for the starter diet (day 1-10), grower diet (day 11-24), and finisher diet (day 24-35) phases as shown in Supplementary Table I, II and III, respectively. Experimental diet fed *ad libitum* to the birds with fresh drinking water through drinkers. The experiment lasted for 35 days. Three inclusion levels (0%, 6% and 12%) of RSM were used, and each RSM level was supplemented without and with protease enzyme ENZ (KEMZYME™ Protease Dry, Kemin Industries, Inc. USA) as single (300 gm/ton of feed) and double (600 gm/ton of feed) dosages.

Growth performance

Weight of birds in each replicate was measured from the time of arrival till the end of trial. The feed intake (FI) of birds was calculated as the difference between the total feed offered and the amount of feed refused.

$$\text{Feed intake} = \text{Total feed offered (g)} - \text{Feed refused (g)}$$

Whereas feed conversion ratio (FCR) was measured for the bird's efficiency in converting feed weight into increased bodyweight as formula given below.

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}$$

Nutrient utilization

Nutrient utilization was determined by using an external marker Acid Insoluble Ash (AIA) Celite added to the experimental diet at 1%. The polythene sheet spread in each pen to collect the droppings. Birds droppings collected after every three hours on day 21 and 35, which were then replicate-wise composited and stored till the analysis for proximate composition. Acid insoluble ash in feed and droppings was determined. The digestibility determined by the following formula.

$$\text{Digestibility Coefficient (\%)} = 100 - \left[100 \times \left(\frac{\% \text{AIA in diet}}{\% \text{AIA in feces}} \right) \times \left(\frac{\% \text{Nutrients in feces}}{\% \text{Nutrients in diets}} \right) \right]$$

Ileal digestibility

Ileal digestibility for crude protein (CP) was determined using following equation.

$$\text{CP digestibility coefficient (\%)} = 100 - \left[100 \times \left(\frac{\% \text{AIA in diet}}{\% \text{AIA in ileal digesta}} \right) \times \left(\frac{\% \text{CP in ileal digesta}}{\% \text{CP in diets}} \right) \right]$$

Carcass characteristics

Birds from each replicate were slaughtered to obtain data on carcass characteristics such as: Dressing percentage, thigh meat yield, drumstick meat yield, liver weight, heart weight, using formula as for: Heart weight % = Heart weight (g)/Live weight (g)×100

Chemical analysis

A sample feed weighing 500 g was collected from each replicate for proximate analysis to determine the dry matter (DM), crude protein (CP), fat, and ash content. DM was measured by drying the feed at 105°C in a hot air oven using AOAC method # 934.01. CP was quantified with automated Kjeldahl analyzer (Kjeltec™ 8400), following AOAC method # 984.13. Fat content was extracted using a Soxhlet apparatus with n-hexane as the solvent, according to AOAC method # 920.39. Finally, ash content was determined by combusting the samples at 550°C in a muffle furnace, using AOAC method # 942.05 (AOAC, 2000).

Statistical analysis

Data obtained was tabulated using Microsoft Excel and further analyzed with ANOVA general linear model using a statistical software Minitab (Version 21.20, © 2022 Minitab, LLC., USA). The results were expressed in Mean±SEM, and the differences were considered significant at $P < 0.05$.

RESULTS

Growth performance in broiler chicken (day 1-14)

Table I shows that RSM level did not affect the BWG from 1 to 7 days but have significant effect ($P > 0.05$), on the BWG, FI and the FCR from 8 to 14 days ($P < 0.05$). The FI was higher at 12% RSM followed by 6% and then 0% RSM, while the FCR value improved at 0% RSM thereafter 6% and then the 12% RSM. Nevertheless, the impact of ENZ was evident on the BWG during both the 1 to 7 days and 8 to 14 days periods ($P < 0.05$), as well as the FI and the FCR from 1 to 7 days ($P < 0.05$). BWG increased in double ENZ afterward single ENZ and then No ENZ, but the FI increased with double ENZ. Moreover, the FCR improved in single and double ENZ. There was an interaction between RSM and ENZ for all performance parameters during both 1 to 7 days and 8 to 14 days ($P < 0.05$), demonstrating that the effect of ENZ depended on the RSM level. At 0% RSM, double ENZ had the highest BWG and the lowest FCR during both 1 to 7 days and 8 to 14 days, while at 6% RSM, single ENZ had the lowest FCR from 8 to 14 days.

Table I. Effect of rapeseed meal with protease supplementation on growth performance in broiler chicken (1-14 days).

RSM (%)	ENZ	Growth performance					
		Day 1-7			Day 8-14		
		BWG (g)	FI (g)	FCR	BWG (g)	FI (g)	FCR
0		181.7	174.6 ^b	0.96 ^b	316.7 ^a	365.5	1.15 ^b
6		183.4	176.0 ^{ab}	0.96 ^b	313.5 ^a	367.1	1.17 ^b
12		180.1	178.7 ^a	0.99 ^a	302.5 ^b	365.7	1.2 ^a
SEM		1.45	0.92	0.007	1.40	2.4	0.01
<i>P</i> -value		0.283	0.015	0.009	0.00	0.889	0.003
	No	179.6	177.9	0.99 ^a	309.1	367.2 ^{ab}	1.18
	Single	182.1	174.8	0.96 ^b	310.6	360.3 ^b	1.16
	Double	183.5	176.7	0.96 ^{ab}	313.1	371.0 ^a	1.18
SEM		1.45	0.92	0.007	1.40	2.4	0.01
<i>P</i> -value		0.17	0.07	0.02	0.140	0.015	0.122
Interaction							
0	No	178.3	175.3 ^{ab}	0.98 ^{abc}	315.9 ^a	367.6	1.16 ^{bc}
	Single	183.0	174.1 ^{ab}	0.95 ^{abc}	316.0 ^a	362.8	1.14 ^{bc}
	Double	184.0	174.3 ^{ab}	0.95 ^{bc}	318.2 ^a	366.3	1.15 ^{bc}
6	No	179.1	179.3 ^a	1.0 ^{ab}	310.2 ^{ab}	367.6	1.18 ^{ab}
	Single	182.3	173.67 ^b	0.94 ^c	214.9 ^a	357.3	1.13 ^c
	Double	188.8	177.2 ^{ab}	0.94 ^c	315.4 ^a	376.3	1.19 ^{ab}
12	No	181.3	179.1 ^a	0.99 ^{abc}	300.9 ^b	366.3	1.21 ^a
	Single	181.1	178.6 ^{ab}	0.99 ^{abc}	300.9 ^b	360.7	1.20 ^a
	Double	177.9	178.5 ^{ab}	1.0 ^a	305.7 ^{ab}	370.1	1.21 ^a
SEM		2.1	2.00	0.004	2.58	4.2	0.0178
<i>P</i> -value		0.06	0.015	0.001	0.00	0.160	0.036

Rapeseed meal at 0%, 6% and 12% were used in different groups, supplemented with and without protease at single and double dosages. RSM: Rapeseed meal; ENZ: Protease enzyme; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio. ^{a, b, c} different letters superscript within group differ significantly with $P < 0.05$

Growth performance in broiler chicken (day 1-35)

Table II shows that with the increasing in RSM levels decreased body weight gain (BWG) ($P < 0.05$), while adding ENZ increased BWG ($P < 0.05$). An interaction between RSM and ENZ for BWG was significant ($P < 0.05$), indicating that ENZ's effect depended on the RSM level. For instance, at 0% RSM, double ENZ had the highest BWG, whereas at 12% RSM, No ENZ had the lowest BWG. However, the feed intake (FI) and feed conversion ratio (FCR) were unaffected by RSM level, but it significantly ($P < 0.05$) influenced by ENZ. Double ENZ resulted in the highest FI and the lowest FCR, while No ENZ showed the lowest FI and the highest FCR.

Table II. Effect of rapeseed meal (RSM) with protease supplementation on growth performance in broiler chicken (1-35 days).

RSM (%)	ENZ	Growth performance after 35 days		
		BWG (g)	FI (g)	FCR
0		2055 ^a	3074	1.49 ^b
6		1996 ^{ab}	3000	1.50 ^b
12		1963 ^b	3034	1.54 ^a
SEM		17.9	28.1	0.01
<i>P</i> -value		0.005	0.202	0.006
	No	1950 ^b	2988 ^b	1.53 ^a
	Single	1978 ^b	3007 ^b	1.52 ^a
	Double	2083 ^a	3114 ^a	1.49 ^a
SEM		17.9	28.1	0.01
<i>P</i> -value		0.000	0.009	0.068
Interaction				
0	No	1988 ^b	3001 ^{ab}	1.51 ^{ab}
	Single	2012 ^{ab}	3038 ^{ab}	1.51 ^{ab}
	Double	2165 ^a	3182 ^a	1.47 ^b
6	No	1944 ^b	2922 ^b	1.50 ^{ab}
	Single	1978 ^b	2966 ^{ab}	1.50 ^{ab}
	Double	2066 ^{ab}	3113 ^{ab}	1.51 ^{ab}
12	No	1920 ^b	3039 ^{ab}	1.58 ^a
	Single	1950 ^b	3016 ^{ab}	1.54 ^{ab}
	Double	2019 ^{ab}	3048 ^{ab}	1.51 ^{ab}
SEM		32.3	48	0.018
<i>P</i> -value		0.002	0.049	0.019

For details of treatment groups, abbreviations, statistical details, see [Table I](#).

Feed digestibility (grower diets) in broiler chicken

[Table III](#) shows that increasing RSM levels decreased the digestibility of dry matter (DM), crude protein (CP) and crude fat (EE) ($P < 0.05$), while adding ENZ increased the digestibility of DM ($P < 0.05$), but not CP and EE. Additionally, the data illustrates the interaction between RSM and ENZ for DM, CP, and EE digestibility ($P < 0.05$), indicating that RSM's impact depends on the ENZ level. Conversely, at 0% RSM, Double ENZ showed better DM values. However, there is no significant ($P > 0.05$) difference between single and double ENZ at 12% RSM for DM value. Moreover, the data shows the increased CP and DM values in RSM 0%, followed by 6%, and then 12%, irrespective of ENZ levels.

Table III. Effect of rapeseed meal (RSM) with protease (ENZ) supplementation on feed digestibility (grower diets) in broiler chicken.

RSM (%)	ENZ	DM (%)	CP (%)	EE (%)
0		83.07 ^a	81.47 ^a	81.95 ^a
6		82.82 ^{ab}	78.62 ^b	79.82 ^b
12		82.37 ^b	77.02 ^b	78.65 ^b
SEM		0.162	0.498	0.379
<i>P</i> -value		0.017	0.00	0.00
	No	81.82 ^b	78.13 ^a	79.91 ^a
	Single dose	83.17 ^a	79.75 ^a	80.74 ^a
	Double dose	83.271 ^a	79.23 ^a	79.76 ^a
SEM		0.162	0.498	0.379
<i>P</i> -value		0.00	0.086	0.170
Interaction				
0	No	82.03 ^c	82.31 ^a	82.11 ^a
	Single dose	83.24 ^{ab}	81.66 ^{ab}	82.14 ^a
	Double dose	83.87 ^a	80.44 ^{bc}	81.59 ^a
6	No	82.46 ^{bc}	78.41 ^d	80.75 ^b
	Single dose	83.147 ^{ab}	79.01 ^{cd}	80.83 ^b
	Double dose	81.46 ^{bc}	78.44 ^d	77.87 ^d
12	No	80.97 ^d	73.68 ^c	76.87 ^e
	Single dose	83.05 ^{ab}	78.58 ^d	79.25 ^c
	Double dose	83.08 ^{ab}	78.79 ^d	79.82 ^c
SEM		0.20	0.306	0.138
<i>P</i> -value		0.00	0.00	0.00

For details of treatment groups, abbreviations, statistical details, see [Table I](#).

Feed digestibility (finisher diets) in broiler chicken

[Table IV](#) shows the effect of RSM with ENZ supplementation on feed digestibility in broiler chickens received on finisher diets. The data presented indicates that the increasing RSM levels led to a decrease in DM, CP, and EE digestibility, as indicated by the comparable statistical values. The results indicated that the protease supplementation enhanced DM, CP, and EE digestibility ($P < 0.05$). However, the higher value of DM was recorded in single after that double and then to No ENZ. Further, there is no significant difference ($P < 0.05$) for CP and EE value between single and double ENZ. Moreover, the CP and EE value was lower ($P > 0.05$) in single when compared to single and double ENZ. The data further illustrates the interaction between RSM and ENZ for each parameter, emphasizing how the effects of RSM depend on the levels of ENZ. Significant differences ($P < 0.05$) were observed in digestibility values of all the treatments of RSM and ENZ.

Table IV. Effect of rapeseed meal with protease supplementation on feed digestibility (finisher diets) in broiler chicken.

RSM (%)	ENZ	DM (%)	CP (%)	EE (%)
0		81.41 ^a	77.26 ^a	82.92 ^a
6		80.65 ^b	74.92 ^{ab}	70.62 ^c
12		80.94 ^{ab}	73.20 ^b	79.26 ^b
SEM		0.196	0.662	0.777
<i>P</i> -value		0.036	0.001	0.000
	No	79.83 ^c	69.79 ^b	71.32 ^b
	Single	82.06 ^a	77.54 ^a	81.177 ^a
	Double	81.11 ^b	78.05 ^a	80.29 ^a
SEM		0.196	0.662	0.777
<i>P</i> -value		0.00	0.000	0.000
Interaction				
0	No	80.36 ^c	74.56 ^d	79.12 ^c
	Single	81.83 ^{ab}	77.00 ^c	83.05 ^b
	Double	82.04 ^a	80.24 ^a	86.58 ^a
6	No	79.96 ^c	68.84 ^c	62.91 ^f
	Single	81.95 ^{ab}	77.09 ^c	77.66 ^d
	Double	80.02 ^c	78.83 ^b	71.29 ^e
12	No	79.17 ^d	65.98 ^f	71.93 ^e
	Single	82.383 ^a	78.53 ^b	82.83 ^b
	Double	81.26 ^b	75.10 ^d	83.01 ^b
SEM		0.157	0.123	0.161
<i>P</i> -value		0.000	0.000	0.000

For details of RSM and enzyme supplementations, abbreviations and statistical details, see Table III.

Carcass parameters in broiler chicken

Table V shows that increasing RSM level decreased the carcass weight ($P < 0.05$), while adding ENZ increased the carcass weight ($P < 0.05$). There was an interaction between RSM and ENZ for carcass weight ($P < 0.05$), indicating that the effect of RSM depended on the ENZ level. In accordance with the results obtained the carcass weight was greater in the single followed by No and then the double ENZ. However, there is no significant difference ($P < 0.05$) observed for thigh, drumstick, and liver weight among all groups. Nevertheless, the heart weight increased in group RSM 12% having no ENZ. Whereas, with ENZ the heart weight increased in group RSM 6% with single dosage of ENZ.

DISCUSSION

Protein is essential for building, repairing, and maintaining tissues and for making enzymes and hormones is the second most vital part of the diet (Yu

and Fukagawa, 2020). RSM is a by-product of rapeseed oil extraction, which is rich in protein, fiber and minerals but also contains anti-nutritional factors (ANF) such as glucosinolates, phytates, tannins and sinapine, that can impair the digestion and utilization of nutrients, affect the thyroid function and cause organ damage in poultry (Negawoldes and Woldes, 2018). Protease is an enzyme that can hydrolyze the protein bound ANF in RSM, and enhance the digestibility and availability of amino acids, especially lysine which is the first limiting amino acid in RSM (Amerah et al., 2015; Tan et al., 2022). Protease can also improve the intestinal morphology and function by increasing the villus height and width reducing the crypt depth and modulating the gut microbiota and immune system in poultry (Alagawany et al., 2021; Amer et al., 2021; Khatun et al., 2022). The present study investigated the effects of RSM and protease supplementation on growth performance, nutrient digestibility, and carcass characteristics on broiler chicken.

Table V. Effect of rapeseed meal with protease supplementation on carcass parameters in broiler chicken.

RSM (%)	ENZ	Carcass (%)	Thigh (%)	Drum-stick (%)	Liver (%)	Heart (%)
0		64.37 ^a	5.507 ^a	4.400 ^a	2.378 ^a	0.433 ^b
6		63.97 ^{ab}	5.327 ^a	4.134 ^a	2.468 ^a	0.471 ^{ab}
12		63.21 ^b	4.986 ^a	4.083 ^a	2.496 ^a	0.537 ^a
SEM		0.245	0.171	0.117	0.068	0.020
<i>P</i> -value		0.010	0.114	0.143	0.452	0.005
	No	63.13 ^b	5.340 ^a	4.365 ^a	2.379 ^a	0.497 ^{ab}
	Single	65.83 ^a	5.569 ^{ab}	4.123 ^a	2.595 ^a	0.518 ^a
	Double	62.59 ^b	4.911 ^b	4.130 ^a	2.367 ^a	0.426 ^b
SEM		0.245	0.171	0.117	0.068	0.020
<i>P</i> -value		0.000	0.037	0.269	0.046	0.011
Interaction						
0	No	63.62 ^{bc}	6.49 ^a	4.73 ^a	2.17 ^b	0.436 ^{ab}
	Single	66.61 ^a	4.88 ^c	4.30 ^{ab}	2.73 ^a	0.408 ^{ab}
	Double	62.86 ^{bc}	5.142 ^c	4.16 ^{ab}	2.23 ^{ab}	0.455 ^b
6	No	63.31 ^{bc}	5.42 ^{bc}	4.57 ^a	2.43 ^{ab}	0.518 ^{ab}
	Single	66.04 ^a	5.78 ^{ab}	3.79 ^b	2.52 ^{ab}	0.494 ^{ab}
	Double	62.56 ^c	4.78 ^c	4.04 ^{ab}	2.45 ^{ab}	0.401 ^b
12	No	62.46 ^c	4.79 ^c	3.79 ^b	2.53 ^{ab}	0.537 ^{ab}
	Single	64.84 ^{ab}	4.81 ^{bc}	4.27 ^{ab}	2.53 ^{ab}	0.607 ^a
	Double	62.34 ^c	5.35	4.04 ^{ab}	2.42 ^{ab}	0.469 ^{ab}
SEM		0.442	0.146	0.146	0.106	0.036
<i>P</i> -value		0.000	0.000	0.003	0.051	0.016

For details of RSM and enzyme supplementations, abbreviations and statistical details, see Table III.

Our current study demonstrates that RSM levels influenced BWG, FI and FCR with significant effects observed across different time periods. ENZ supplementation remarkably impacted BWG, FI and FCR indicating its potential to improve broiler performance. Additionally, the interaction between RSM and ENZ highlights the subtle effects of RSM depending on the ENZ level, indicating prospective optimization approaches for broiler diets. Broiler performance improved with 0.05% supplementation of protease (Al-Harathi, 2017). However, some other studies found no or variable effects of RSM and protease on the performance, digestibility and carcass weight of broiler chickens (Kendala *et al.*, 2020; Konkol *et al.*, 2024; Wu *et al.*, 2020). Body weights and FCR improved with protease supplementation on day 35 regardless source as compared to non-supplementation groups (Toghyani *et al.*, 2017).

Current study reveals that increasing RSM levels reduce the digestibility of DM, CP and EE while ENZ supplementation enhances DM digestibility. A comparable study by Rada *et al.* (2016) illustrates that increasing the amount of rapeseed meal in the diet had no influence on amino acids digestibility even when exogenous protease was added. The study also highlights an interaction between RSM and ENZ, suggesting RSM depends on ENZ levels with specific observations regarding DM values at different RSM levels and ENZ supplementation. On the contrary, Cowieson and Adeola (2005) stated that digestible energy values in birds' intestines can be enhanced by adding protease enzyme in the diet of broiler chicken as compared to the control group diet in which nutritional requirement fulfills without adding protease supplementation. However, Rostagno *et al.* (2000) reported that 3% protein digestibility can be enhanced by supplementation of protease in corn-soybean meal-based diets.

Supplementing broiler diets with protease enzymes in RSM enhances nutrient digestibility by breaking down complex proteins and anti-nutritional factors present in the feed. Protease enzymes catalyze the hydrolysis of peptide bonds in proteins, leading to the release of free amino acids and peptides which are easily absorbed by the bird's digestive system. This breakdown of proteins and anti-nutritional factors such as glucosinolates and phytic acid improves the availability of essential nutrients for absorption in the intestine. As a result, broiler chickens can efficiently utilize the nutrients present in rapeseed meal, leading to enhanced nutrient digestibility.

The existing research shows that increasing RSM level decreased the carcass weight ($P < 0.05$), while adding ENZ increased the carcass weight ($P < 0.05$). In accordance with the results obtained the carcass weight

was greater in the single followed by No and then the Double ENZ. However, there is no significant difference ($P < 0.05$) observed for thigh, drumstick and liver weight among all groups. Nevertheless, the increased heart weight noticed in RSM 12% without ENZ. Whereas, with a single protease dose, heart weight increased in RSM 6%. A similar study by Fu *et al.* (2021) reports that dietary RSM up to 16% did not affect the carcass weight, yield, or organ weights of geese. The study also indicates an interaction between RSM and ENZ, implying that carcass performance depends on the levels of RSM and ENZ, with specific observations regarding carcass weight at different RSM levels and ENZ supplementation. Conversely, Thomke *et al.* (1983) reported that dietary enzymes improved carcass weight and dressing percentage in broilers fed high content of RSM. However, the effects of RSM and enzyme supplementation on poultry carcass quality remain inconsistent across numerous studies. Some research suggests positive outcomes, while others indicate minimal or no improvement, highlighting the variability in response depending on factors such as enzyme type, dosage, and dietary composition. Some studies have shown that RSM can reduce the fat content and increase the protein content of poultry meat (Biesek *et al.*, 2020; Skugor *et al.*, 2019), while others have found no significant differences in meat quality parameters between RSM and soybean meal diets (Fu *et al.*, 2021).

It can be speculated that protease enzymes can degrade anti-nutritional factors (ANFs) present in the diet, thereby reducing their negative impact and enhancing nutrient availability and absorption. This increased nutrient efficiency supports better growth and development, contributing to higher carcass weight in poultry. Additionally, by improving gut health and reducing nutrient excretion, protease enzymes further optimize the overall performance and productivity of broiler chickens.

CONCLUSION

In conclusion, protein digestibility of rapeseed meal (RSM) can be improved by protease supplementation. Double dosage of protease showed positive impact on protein content in RSM thereby directly improved bird performance and protein digestibility. Moreover, bird performance and protein digestibility decreased in negative control treatments as compared to positive. Including RSM at a level of 12% affected negatively on overall performance and health of bird because of the available anti-nutritional factors. Therefore, RSM inclusion at 6% with protease supplementation in broiler diet improved growth performance, nutrient digestibility, and carcass characteristics in broiler chicken.

DECLARATIONS

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IRB approval

The proposal of present study received approval from the Board of Advanced Studies and Research (BASR) as Ref # DAS/2465) during year 2023.

Ethical approval

This research was conducted at Sindh Agriculture University, Tandojam, Pakistan in accordance with the guidelines of animal care and slaughtering, prior to approval by the Institutional Ethical Committee (Approval No.: DAS/2465/of 2023; Dated: 13-10-2023).

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20260404013256>

Generative AI and AI-assisted technology statement

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

Statement of conflict of interest

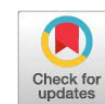
The authors have declared no conflict of interest.

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Supplementary Material

Impact of Rapeseed Meal at Various Levels with Protease Supplementation on Growth Performance, Nutrient Digestibility, and Some Carcass Characteristics in Broiler Chicken

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Supplementary Table I. Ingredients and chemical composition of starter diet (day 1-10).

Ingredients	¹ T1	² T2	³ T3	⁴ T4	⁵ T5	⁶ T6	⁷ T7	⁸ T8	⁹ T9
Corn	58.01	57.99	57.97	54.26	54.24	54.22	50.19	50.17	50.15
Soybean meal	6	6	6	6	6	6	6	6	6
Rapeseed meal	0	0	0	6	6	6	12	12	12
Rice polish	5	5	5	5	5	5	5	5	5
Canola meal	8	8	8	8	8	8	8	8	8
Guar meal	5	5	5	5	5	5	5	5	5
Sunflower meal	4	4	4	4	4	4	4	4	4
Fish meal	9	9	9	5.50	5.50	5.50	2.50	2.50	2.50
PBM ¹⁰	3	3	3	3	3	3	3	3	3
Soya oil	0.91	0.91	0.91	2.03	2.03	2.03	3.25	3.25	3.25
DCP ¹¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Limestone	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Soda bi-carb	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.22	0.22	0.22	0.34	0.34	0.34	0.19	0.19	0.19
Methionine	0.11	0.11	0.11	0.12	0.12	0.12	0.12	0.12	0.12
Premix ¹²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Protease	0	0.03	0.06	0	0.03	0.06	0	0.03	0.06
Total	100	100	100	100	100	100	100	100	100

Table continues on next page.....

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0030-9923/2026/0001-0001 \$ 9.00/0



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Ingredients	¹ T1	² T2	³ T3	⁴ T4	⁵ T5	⁶ T6	⁷ T7	⁸ T8	⁹ T9
Chemical composition									
Crude protein	21	21	21	21	21	21	21	21	21
ME (kcal/kg) ¹³	3000	3000	3000	3000	3000	3000	3000	3000	3000
Crude fiber	3.74	3.74	3.74	4.32	4.32	4.32	4.90	4.90	4.90
Ether extract	5.53	5.53	5.53	6.28	6.28	6.28	7.15	7.15	7.15

¹Rapeseed meal 0% without protease enzyme, ²Rapeseed meal 0% with standard dose protease enzyme, ³Rapeseed meal 0% with double dose protease enzyme, ⁴Rapeseed meal 6% without protease enzyme, ⁵Rapeseed meal 6% with standard dose protease enzyme, ⁶Rapeseed meal 6% with double dose protease enzyme, ⁷Rapeseed meal 12% without protease enzyme, ⁸Rapeseed meal 12% with standard dose protease enzyme, ⁹Rapeseed meal 12% with double dose protease enzyme, ¹⁰Poultry by-product meal, ¹¹Di-calcium phosphate, ¹²Vitamin and mineral premix, ¹³Metabolizable energy.

Supplementary Table II. Ingredients and chemical composition of grower diet (day 11-24).

Ingredients	¹ T1	² T2	³ T3	⁴ T4	⁵ T5	⁶ T 6	⁷ T7	⁸ T8	⁹ T9
Corn	61.31	61.29	61.27	57.46	57.44	57.42	53.6	53.58	53.56
Soybean meal	4	4	4	4	4	4	4	4	4
Rapeseed meal	0	0	0	6	6	6	12	12	12
Rice polish	5	5	5	5	5	5	5	5	5
Canola meal	5	5	5	5	5	5	5	5	5
Guar meal	5	5	5	5	5	5	5	5	5
Sunflower meal	4	4	4	4	4	4	4	4	4
Fish meal	10	10	10	6.65	6.65	6.65	3.32	3.32	3.32
PBM ¹⁰	3	3	3	3	3	3	3	3	3
Soya oil	1.43	1.43	1.43	2.59	2.59	2.59	3.74	3.74	3.74
DCP ¹¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Limestone	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Soda bi-carb	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.39	0.39	0.39	0.42	0.42	0.42	0.45	0.45	0.45
Methionine	0.12	0.12	0.12	0.13	0.13	0.13	0.14	0.14	0.14
Premix ¹²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Protease	0	0.03	0.06	0	0.03	0.06	0	0.03	0.06
Total	100	100	100	100	100	100	100	100	100
Chemical composition									
Crude protein	20	20	20	20	20	20	20	20	20
ME (kcal/kg) ¹³	3100	3100	3100	3100	3100	3100	3100	3100	3100
Crude fiber	3.36	3.36	3.36	3.94	3.94	3.94	4.52	4.52	4.52
Ether extract	6.17	6.17	6.17	6.96	6.96	6.96	7.75	7.75	7.75

For details of treatment groups, abbreviations, superscripts, see [Supplementary Table I](#).

Supplementary Table III. Ingredients and chemical composition of finisher diet (day 24-35).

Ingredients	¹T1	²T2	³T3	⁴T4	⁵T5	⁶T6	⁷T7	⁸T8	⁹T9
Corn	64.09	64.07	64.05	60.13	60.11	60.09	56.05	56.03	56.01
Soybean meal	8	8	8	8	8	8	8	8	8
Rapeseed meal	0	0	0	6	6	6	12	12	12
Rice polish	5	5	5	5	5	5	5	5	5
Canola meal	3	3	3	3	3	3	3	3	3
Guar meal	3	3	3	3	3	3	3	3	3
Sunflower meal	2	2	2	2	2	2	2	2	2
Fish meal	9.18	9.18	9.18	6	6	6	3	3	3
PBM ¹⁰	2	2	2	2	2	2	2	2	2
Soya oil	2.41	2.41	2.41	3.60	3.60	3.60	4.82	4.82	4.82
DCP ¹¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Limestone	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Soda bi-carb	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lysine	0.42	0.42	0.42	0.37	0.37	0.37	0.22	0.22	0.22
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.16	0.16	0.16
Premix ¹²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Protease	0	0.03	0.06	0	0.03	0.06	0	0.03	0.06
Total	100	100	100	100	100	100	100	100	100
Chemical composition									
Crude protein	19	19	19	19	19	19	19	19	19
ME (kcal/kg) ¹³	3200	3200	3200	3200	3200	3200	3200	3200	3200
Crude fiber	2.70	2.70	2.70	3.28	3.28	3.28	3.86	3.86	3.86
Ether extract	6.98	6.98	6.98	7.81	7.81	7.81	8.68	8.68	8.68

For details of treatment groups, abbreviations, superscripts, see [Supplementary Table I](#).