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Effects of Dietary Protein Levels Enriched with Subtilisin Protease on the Performance and Nutrients Utilization of Broiler Chicks

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Key words
Subtilisin enzyme, Broiler, AME, Protein, Feed conversion ratio

ABSTRACT

The study was conducted to investigate the effect of the minimum level of dietary protein and subtilisin protease enzyme on the performance and nutrient utilization in broiler chicks. Different levels of proteins with and without subtilisin protease enzyme were evaluated on the overall performance, carcass yield, and nutrient utilization. For this purpose, 3 proteins and 2 protease levels were arranged in a 3x2 factorial design and each group was divided into 4 replicates of 10 chicks each. All parameters were calculated significantly different. The interaction effect of ($P_{21.5}$) and enzyme gave the highest feed intake and weight gain value among all the interactions. The highest crude protein was noted for protein level (P19.5) and maximum crude protein was obtained with enzyme treatment. Protein level ($P_{19.5}$) and treatment having enzyme gave the highest AME. It was noticed that the lowest level of protein gave the highest FCR while the enzyme effect showed decreased FCR. The highest live weight, carcass weight, and carcass yield were recorded with the treatment having the highest level of dietary protein and enzyme. It is concluded that dietary protein in combination with subtilisin protease enzyme has a positive effect on the overall performance and nutrient utilization in the broiler.

INTRODUCTION

Pakistan is ranked 11th in the total meat-producing countries of the world where the poultry industry contributed about 35% of the total meat production (Anonymous, 2020-2021). This situation revealed the future potential and dynamic importance of the broiler farming industry in Pakistan. Modern broiler chickens have been modified genetically to procure quick and high quality meat in the shortest possible time. As the growth to marketing age has been progressively shortened, the efficient feed utilization for optimum broiler growth production is becoming more pronounced and important in commercial broiler production. To enhance growth production, optimized nutrient utilization, especially of dietary protein and amino acids, are of utmost importance. However, the higher price of protein-derived feed ingredients compels the nutritionist for the inclusion of low-quality feed ingredients. It not only increases health risks but has lessened the profit margin and elevated environmental pollution (Alagawany *et al*., 2017; Patterson and Lorenz, 1996, 1997). The presence of anti-nutrients in the low-quality feed further exacerbates the situation by making chelation thereby declining the availability of nutrients for poultry (Ravindran *et al*., 2006). Its nutritive worth can be improved through the use of exogenous enzymes. For the last 5 decades, enzymes have played a vital role in the poultry feed industry by augmenting the nutritive worth of the feed ingredients. They are incorporated into poultry diets to reduce feed costs without

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compromising weight gain and feed efficiency (Walters, 2019; Miles *et al*., 1984; Tahir *et al*., 2008; Woyengo *et al*., 2010; Selle and Ravindran, 2007). A lot of studies have been conducted on the broiler diet on exogenous enzyme supplementation (Walters, 2019; Tahir *et al*., 2005; 2006; Khattak *et al*., 2006), due to which growth of broiler chicks was improved and the available nutrients are well utilized in the diets. Exogenous supplementation of enzymes in the diet of broiler chick has got great attention in respect of good utilization of a low protein diet (Cowieson and Adeola, 2005). Although the importance of enzymes in poultry nutrition is well explained by the researchers yet the efficiency of proteases in low-density diets on the overall performance of broiler chicks is not fully described in the literature.

Protease is a protein-digesting enzyme that acts on the cereal part of the diet and changes complex and larger molecules into small size digestible molecules (Bedford *et al*., 2022). Protease convert compound molecule of protein into smaller peptides and amino acids molecules which are absorbed easily from the GIT tract and revealed improved consumption of nutrients (Bedford *et al*., 2022; Angel *et al*., 2011). Fru *et al*. (2011) demonstrated that exogenous protease had the potential of improving the performance of broiler chick by increasing the energy and protein digestibility. Enzymes are secreted for the digestion and metabolism of nutrients. However, at an early age due to an immature gut system the enzyme especially protease is not enough to efficiently digest the available substrate. Thus dietary protease is supplemented in the diets to assist the internal enzymes in enhancing protein digestion. Recently, bacilisin protease had shown prompt response in low-density diets in supporting the optimum digestion of the available substrate (Angel *et al*., 2011). It was expected that the subtilisin protease used in this study will assist the broiler gut ecosystem by improving the digestive enzymatic activities and will compensate for the performance of broiler chicks on low-density diets. For this purpose boiler diet of different protein levels was subjected to subtilisin protease enzyme to investigate the optimum protein level enriched with protease without compromising the nutrient utilization and performance in broiler chicks.

MATERIALS AND METHODS

Rearing and management practices

This study was conducted at Poultry Research Center Mansehra under the guideline of the Department of Animal Nutrition, Agriculture University, Peshawar, KPK. A total of 240 broiler birds were used for the experimental purpose and were distributed in six dietary treatments with four replicates containing 10 birds each. All the required arrangements for the raring of the birds were carried out.

Experimental design and feeding practices

A factorial design (3 x 2) containing 3 dietary protein levels supplemented with 2 levels of an enzyme (subtilisin protease) was used. Three experimental diets were formulated having CP levels of 17.5, 19.5, and 21.5% to meet the lowest requirements (NRC, 1994). Two levels of protease enzyme (0 and 30,000 IU/kg of feed) were supplemented to these diets. The total period of the experiment was 3 weeks and experimental diets were used for chicks from 7 to 21 days. Experimental diet composition is shown in Table I.

Measurement of growth traits

Feed intake (FI), body weight gains (BWG), and feed conversion ratio (FCR) were calculated at the end of each week. The BWG was calculated by subtracting the initial body weight from the final BWG. Feed intake was determined by subtracting the feed used from the feed offered and FCR was calculated by dividing the feed intake by weight gain.

Dressing percentage

At the end of the experiment (day 21), 3 birds per replicate were slaughtered and the dressing percentage was calculated through the formula:

$$\text{Dressing \%} = \frac{\text{Dress bird weight (g)}}{\text{Live body weight (g)}} \times 100$$

Nutrient digestibility

To determine the nutrient digestibility, five birds/ replicate of the same weight was transferred to the metabolic cages at the age of 19 days and provided the calculated amount of feed. Celite (Sigma Aldrich) was used in feed at 1% as an inert digestibility marker. Total fecal material was collected two times a day (morning and evening) from each cage for the next two days. On day 21 of the experiment, three birds per replicate were randomly selected, slaughtered and ileal digesta was collected and samples were analyzed for dry matter, crude protein, nitrogen-free extract, crude fat, and ash by the method as described by (Shuaib et al., 2020). The feed and feces gross energy (G.E) were calculated by bomb calorimeter and the Nitrogen content of the feed and feces was calculated by the Kjeldahl apparatus (Shuaib et al., 2022). The following formula was used for nutrient digestibility.

Apparent digestibility (%) = 100 – ((Concentration of marker in feed/ concentration of marker in digesta) × (Concentration of nutrient in digesta/ Concentration of nutrient in feed) × 100).

Table I. Composition and calculated analysis of experimental diets.

Ingredient (%)	Normal diet	Low density diet	
	$CP_{21.5}$	$CP_{19.5}$	$CP_{17.5}$
Corn, grain	55.8	59.4	66.0
Soybean meal -44%	16.0	11.0	8.00
Corn gluten M-30%	3.00	2.59	4.44
Canola M	8.00	7.00	7.00
Gluten meal-60	3.00	3.50	1.21
Vegetable oil	2.07	2.00	1.00
Poultry by-product M	3.00	3.00	2.00
Sunflower M	5.00	6.00	5.74
Bone meal	1.00	1.00	1.00
Limestone	1.01	0.86	1.00
Dical. phos.	0.65	1.00	1.00
Common salt	0.41	1.75	0.45
DL-methionine	0.13	0.17	0.28
L-lysine HCL	0.69	0.40	0.55
Threonine	0.05	0.09	0.18
Vit min premix	0.12	0.12	0.12
Total	100	100	100
Calculated composition			
ME (kcal/g)	2.95	2.95	2.95
CP (%)	21.5	19.5	17.5
Available P (%)	0.45	0.45	0.45
Lys (%)	1.15	1.15	1.15
Met (%)	0.52	0.54	0.59
Met+Cys (%)	0.90	0.90	0.90
Thr (%)	0.82	0.80	0.80

Statistical analysis

The analysis of variance (ANOVA) technique was used for the data analysis through the procedure of the general linear model of (SAS, 2006). Duncan test was used for each treatment means. The data were considered significant for the value of probability less than 0.05 ($P<0.05$).

RESULTS

Growth traits and nutrient digestibility

Results regarding the feed intake (FI) and weight gain (WG) are presented in Table II. The highest level of protein gave the highest FI value and the least body weight was recorded for ($P_{17.5}$) protein level. The highest level of protein gave the highest weight gain which is not statistically different from the protein level ($P_{19.5}$). The interaction effect showed that the treatment combination of $P_{21.5}$ and enzyme gave the highest weight gain among all the interactions. The result related to feed conversion ratio (FCR) is shown in Table III. The highest FCR was calculated for the lowest level of protein ($P_{17.5}$) and the results for ($P_{19.5}$) and ($P_{21.5}$) levels were statistically similar. Results related to the enzyme effect showed decreased FCR. The interaction effect showed that the treatment combination of ($P_{17.5}$×O) gave the highest FCR among all the interactions. Results regarding live weight, carcass weight, carcass yield, and nutrient digestibility are shown in Table IV. It was observed that an increase in the level of protein resulted in increased live weight and the overall mean value for protein level ($P_{21.5}$) gave the

Table II. Effect of dietary protein and supplementation of protease on feed intake and weight gain.

Parameters	Days	Protein level	Enzyme level		Mean
			0	E	
Feed intake (g)	7-14	$CP_{17.5}$	513	522^{ab}	518^{b}
		$CP_{19.5}$	543^{a}	547^{a}	545^{a}
		$CP_{21.5}$	535^{ab}	544^{a}	539^{a}
		Mean	531^{b}	538^{a}	--
	15-21	$CP_{17.5}$	673^{ab}	707^{a}	690^{ab}
		$CP_{19.5}$	659^{ab}	639^{b}	649^{b}
		$CP_{21.5}$	696^{ab}	705^{a}	701^{a}
		Mean	676^{a}	684^{a}	--
	7-21	$CP_{17.5}$	1186	1230	1208^{b}
		$CP_{19.5}$	1202	1187	1194^{c}
		$CP_{21.5}$	1231	1249	1240^{a}
		Mean	1206^{b}	1222^{a}	--
Weight gain (g)	7-14	$CP_{17.5}$	280^{c}	310^{bc}	295^{b}
		$CP_{19.5}$	332^{ab}	348^{a}	340^{a}
		$CP_{21.5}$	328^{ab}	357^{a}	342^{a}
		Mean	313^{b}	338^{a}	--
	15-21	$CP_{17.5}$	374^{d}	413^{bc}	393^{c}
		$CP_{19.5}$	403^{cd}	433^{abc}	418^{b}
		$CP_{21.5}$	435^{ab}	450^{a}	443^{a}
		Mean	404^{b}	432^{a}	--
	7-21	$CP_{17.5}$	653^{d}	723^{c}	688^{b}
		$CP_{19.5}$	736^{bc}	781^{b}	758^{a}
		$CP_{21.5}$	760^{abc}	805^{a}	783^{a}
		Mean	716^{b}	770^{a}	--

Each value in the table is a mean of three replicate. Statistically significant difference ($P\leq0.05$) is not there in the means followed by the same letters in the same row or column (Fisher's protected LSD test).

Table III. Effect of dietary protein and supplementation of protease on FCR.

Days	Protein level	Enzyme level		Mean
		0	E	
7-14	$CP_{17.5}$	1.84^{a}	1.69^{b}	1.77^{a}
	$CP_{19.5}$	1.64^{bc}	1.58^{bc}	1.61^{b}
	$CP_{21.5}$	1.65^{bc}	1.53^{c}	1.59^{b}
	Mean	1.71^{a}	1.60^{b}	--
15-21	$CP_{17.5}$	1.81^{a}	1.72^{ab}	1.76^{a}
	$CP_{19.5}$	1.63^{bc}	1.48^{d}	1.56^{b}
	$CP_{21.5}$	1.60^{bcd}	1.57^{cd}	1.58^{b}
	Mean	1.68^{a}	1.59^{b}	--
7-21	$CP_{17.5}$	1.82^{a}	1.71^{b}	1.76^{a}
	$CP_{19.5}$	1.64^{bc}	1.52^{e}	1.58^{b}
	$CP_{21.5}$	1.62^{cd}	1.55^{de}	1.59^{b}
	Mean	1.69^{a}	1.59^{b}	--

Each value in the table is a mean of three replicate. Statistically significant difference ($P \leq 0.05$) is not there in the means followed by the same letters in the same row or column (Fisher's protected LSD test).

Table IV. Effect of dietary protein and enzyme protease on broiler live weight, carcass weight, carcass yield and nutrient digestibility (Day-21).

Parameters	Protein level	Enzyme level		Mean
		0	E	
Live weight (g)	$CP_{17.5}$	803^{d}	873^{c}	838^{b}
	$CP_{19.5}$	886^{bc}	931^{ab}	908^{a}
	$CP_{21.5}$	910^{abc}	955^{a}	933^{a}
	Mean	866^{b}	920^{a}	--
Carcass weight (g)	$CP_{17.5}$	519^{d}	584^{c}	551^{b}
	$CP_{19.5}$	604^{bc}	659^{a}	631^{a}
	$CP_{21.5}$	626^{b}	669^{a}	647^{a}
	Mean	583^{b}	637^{a}	--
Carcass yield %	$CP_{17.5}$	65.2^{f}	67.4^{e}	65.7^{b}
	$CP_{19.5}$	68.1^{d}	71.1^{a}	69.3^{a}
	$CP_{21.5}$	69.3^{c}	70.3^{b}	69.4^{a}
	Mean	67.0^{b}	69.2^{a}	--
Crude protein %	$CP_{17.5}$	70.4^{d}	72.2^{b}	71.0
	$CP_{19.5}$	71.2^{c}	72.4^{a}	71.7
	$CP_{21.5}$	71.1^{c}	71.7^{b}	71.4
	Mean	70.8^{b}	72.0^{a}	--
AME (Kcal/kg)	$CP_{17.5}$	2802^{c}	2893^{b}	2848^{b}
	$CP_{19.5}$	2866^{b}	2949^{a}	2908^{a}
	$CP_{21.5}$	2859^{b}	2910^{ab}	2884^{ab}
	Mean	2843^{b}	2917^{a}	--

Each value in the table is a mean of three replicate. Statistically significant difference ($P \leq 0.05$) is not there in the means followed by the same letters in the same row or column (Fisher's protected LSD test).

highest value for live weight. The lowest live weight was noticed in the lowest level of protein ($P_{17.5}$). The treatments having enzymes gave higher live weight as compared to the treatment without enzymes. The interaction effect significantly affected the live weight and the highest value was noticed in the treatment combination of ($P_{21.5}\times E$) while the lowest value for live weight was in the case of ($P_{17.5}$). Protein level ($P_{21.5}$) gave the highest value for carcass weight. And higher carcass weight was seen in the case of treatment having enzyme and lower was in case of no enzyme. The highest carcass weight value was observed in the treatment combination of ($P_{21.5}\times O$) while the lowest interaction effect was noted in the treatment combination of ($P_{17.5}\times O$). It was observed that an increase in the level of protein resulted in increased carcass yield and the overall mean value for protein level ($P_{21.5}$) gave the highest value for carcass weight. The lowest carcass yield was noticed in the lowest level of protein and treatment having enzyme gave higher carcass yield as compared to the treatment without enzyme. The interaction effect also significantly affects the carcass yield and the highest carcass yield was noticed in the treatment combination of ($P_{19.5}\times E$) while the lowest value was in the case of ($P_{17.5}$). The results obtained for percent CP digestibility were significantly different. The interaction of different protein and enzyme levels was significantly different from each other and the highest crude protein was noted for protein level ($P_{19.5}$) followed by ($P_{21.5}$) level. The maximum percent crude protein was obtained with an enzyme treatment and the least was observed in the case without an enzyme. Protein level ($P_{19.5}$) gave the highest AME value and a higher AME value was seen in the case of treatment having enzyme and lower was in case of no enzyme.

DISCUSSION

The findings of this study revealed that the protein levels as well as enzyme supplementation significantly affect nutrient utilization and other studied attributes. It was noticed that the interaction effect of different protein levels and enzyme supplementation was significant for most of the parameters. The highest level of protein gave the highest feed intake value and the least body weight was recorded when protein level ($P_{17.5}$) was added to the feed. These results can be supported by the findings of Tabeidian *et al.* (2005) who concluded that the birds fed the diet with higher dietary protein concentration exhibited greater ($P<0.05$) growth rate, and feed intake than those fed lower dietary protein concentrations. The interaction effect showed that the treatment combination of ($P_{21.5}\times E$) gave the highest weight gain among all the interactions which is in line with the finding of Fru *et al.* (2011) who reported that the broilers fed with a high protein supplemented diet have 7.5 % more weight (P<0.05) related to those birds which served low protein (LP) feed. These results are also in line with the work of Ghazi *et al*. (2002) who assessed two types of enzyme proteases produced from *Aspergillus* and *Bacillus* and reported enhancements in bird body weight (BW) and FI only with protease-treated soybean meal diet fed to bird produced from *Aspergillus*. These increases in feed intake could be due to improved amino acid balance in a high-protein diet. Enhancements in body weight might be due to the increases in FI because of progress in nitrogen digestibility and retention time in GIT and augmented passage rate of digesta. The highest level of dietary protein and enzyme supplementation gave the lowest FCR value which is in agreement with the result of Yuan *et al*. (2017) who evaluate the effect of the enzyme on FCR and reported that the feed to gain ratio (FGR) was significantly improved by supplementation with non-starch polysaccharide (NSP) enzymes or NSP enzyme combined with 40 or 80 mg/kg protease compared to the control diet (P<0.05).

The highest carcass weight value was observed in the treatment combination (P21.5×O) and the lowest interaction effect was noted in the treatment combination of (P17.5×O). These results are in agreement with the results of Rada *et al*. (2013) who reported that carcass characteristics were improved in the broiler with the supplementation of protease enzyme. Freitas *et al*. (2011) also concluded the significant effect of dietary protein and enzyme supplementation on carcass weight. The highest carcass yield was noticed in treatment combination ($P_{19.5}$×E) while the lowest value in the case of (P17.5×0) and this results is supported by the finding of Rada *et al*. (2014) who described that protease enzyme significantly improved the carcass yield as compared to control group. The maximum percent crude protein was obtained with an enzyme treatment and the least CP% was observed in cases without enzyme. These results are supported by the work of Angel *et al*. (2011) who reported that the percent crude protein in broiler was improved by the supplementation of protease enzyme. The highest AME value was observed in the treatment combination of (P19.5×O) and the lowest interaction effect was noted in the treatment combination of (P17.5×O) These results are in line with the result of Freitas *et al*. (2011) who find out the result of exogenous protease enzyme added in the broiler diet and revealed that the use of protease enhanced feed efficiency and AME values of diet as well as digestibility of fat and crude protein.

CONCLUSIONS AND RECOMMENDATIONS

The protein level $P_{21.5}$ enriched with subtilisin protease significantly improved the growth performance, carcass parameters and digestibility of crude protein, and apparent metabolizable energy. Generally, the best effect was found at the highest level of dietary protein enriched with enzyme (Subtilisin). Poultry farmers can obtain broilers of better quality by feeding protein level ($P_{21.5}$) enriched with subtilisin enzyme. Further research on how the subtilisin enzyme supplementation in diet influenced growth performance is recommended.

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Animal welfare statement

The experiment was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Science, The University of Agriculture Peshawar, Pakistan.

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IRB approval

The study was approved by the Board of Study (BOS) conducted at Department of Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar (on May, 2018).

Ethical statement

The study was approved by the Animal Welfare and Care Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, and all the measures and tools were considered to minimize pain and discomfort of birds during conduction of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Alagawany, M., Attia, A.I., Ibrahim, Z.A., Mahmoud, R.A., and El-Sayed, S.A., 2017. The effectiveness of dietary sunflower meal and exogenous enzyme on growth, digestive enzymes, carcass traits and blood chemistry of broilers. *Environ. Sci. Poll. Res.*, **24**: 12319–12327. https://doi.org/10.1007/s11356-017-8934-4

Angel, R., Saylor, W., Vieira, S.L., and Ward, N., 2011. Effects of a mono-component protease on performance and protein utilization in 7 to 22 days old broiler chickens. *Poult. Sci.*, **90:** 2281-2286. https://doi.org/10.3382/ps.2011-01482

Anonymous, 2020-21. *Pakistan economic survey 2020-21*. Finance and Economic Affairs Division, Ministry of Finance, Govt. of Pakistan, Islamabad. Pakistan.

Bedford, M.R., and Apajalahti, J.H., 2022. The role of feed enzymes in maintaining poultry intestinal health. *J. Sci. Fd. Agric*., **102**: 1759-1770. https://doi.org/10.1002/jsfa.11670

Cowieson, J., and Adeola, O., 2005. Carbohydrases, protease and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poult. Sci*., **84**: 1860-1867. https://doi.org/10.1093/ps/84.12.1860

Economic Survey of Pakistan, 2013-14. *Economic advisor's wing*. Ministry of Finance, Government of Pakistan, Islamabad.

Freitas, D.M., Vieira, S.L., Angel, C.R., Favero, A., and Maiorka, A., 2011. Performance and nutrient utilization of broilers fed diets supplemented with a novel mono-component protease. *J. appl. Poult*. Res., **20**: 322–334. https://doi.org/10.3382/japr.2010-00295

Fru-nji, F., Kluenter, A.M., Fischer, M., and Pontoppidan, K., 2011. A feed serine protease improves broiler performance and energy digestibility. *J. Poult. Sci.*, **48**: 239-246. https://doi.org/10.2141/jpsa.011035

Ghazi, S., Rooke, J.A., Galbraith, H., and Bedford, M.R., 2002. The potential for the improvement of the nutritive value of soya-bean meal by different proteases in broiler chicks and broiler cockerels. *Br. Poult. Sci*., **43**: 70-77. https://doi.org/10.1080/00071660120109935

Khattak, F.M., Pasha, T.N., Hayat, Z., and Mahmud, A., 2006. Enzymes in poultry nutrition. *J. Anim. Poult. Sci*., **16**: 464-470.

Miles, R.D., Janky, D.M., and Harms, R.H., 1984. Virginiamycin and broiler performance. *Poult. Sci*., **63**: 1218–1221. https://doi.org/10.3382/ps.0631218

National Research Council, 1994. *Nutrient requirements of poultry*. National Academy Press, Washington, DC.

Patterson, P.H., and Lorenz, E.S., 1996. Manure nutrient production from commercial white Leghorn hens. *J. appl. Poult. Res*., **5**: 260–268. https://doi.org/10.1093/japr/5.3.260

Patterson, P.H., and Lorenz, E.S., 1997. Nutrients in manure from commercial white Leghorn pullets. *J. appl. Poult. Res*., **6**: 247–252. https://doi.org/10.1093/japr/6.3.247

Rada, V., Foltyn, M., Lichovníková., M., and Musilová, A., 2013. Effects of protease supplementation of low protein broiler diets on growth parameters and carcass characteristic. *Mendelnet*, 268-272. ID: 28080520.

Rada, V., Lichovníková, M., and Foltyn, M., 2014. The effect of serine protease on broiler growth and carcass quality. *Acta Fytotech. Zoot.*, **17**: 87-89. https://doi.org/10.15414/afz.2014.17.03.87-89

Ravindran, V., Morel, P.C.H., Partridge, G.G., Hruby, M., and Sands, J.S., 2006. Influence of an *Escherichia coli* derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. *Poult. Sci*., **85**: 82–89. https://doi.org/10.1093/ps/85.1.82

SAS Institute, 2006. *SAS user's guide: Statistics*. Version 9.1.3 ed. SAS Inst. Inc., Cary, NC.

Selle, P.H., and Ravindran, V., 2007. Microbial phytase in poultry nutrition. *Anim. Feed. Sci. Technol*., **135**: 1-41. https://doi.org/10.1016/j.anifeedsci.2006.06.010

Shuaib, M., Hafeez, A., Chand, N., and Tahir, M., 2022. Effect of dietary inclusion of soybean hull on production performance and nutrient digestibility during peak egg production period with different phases in laying hens, *Pakistan J. Zool.,* **9**: 2022. https://doi.org/10.17582/journal.pjz/20211105091115

Shuaib, M., Ullah, N., Hafeez, A., Alhidary, I.A., Abdelrahman, M.M., and Khan, R.U., 2020. Effect of dietary supplementation of wild Cumin (Bunium persicum) seeds on performance, nutrient digestibility and circulating metabolites in broiler chicks during the finisher phase. *Anim. Biotechnol*., 33: 871-875. https://doi.org/10.1080/10495398.2020.1844222

Tabeidian, A., Sadeghi, G.H., and Pourreza, J., 2005. Effect of dietary protein levels and soybean oil supplementation on broiler performance. *Int. J. Poult. Sci.*, **4**: 799-803. https://doi.org/10.3923/ijps.2005.799.803

Tahir, M., Saleh, F., Ohtsuka, A., and Hayahsi, K., 2005. Synergistic effect of cellulase and hemicellulase on nutrient utilization and performance in broilers fed a corn-soybean meal diet. *Anim. Sci. J*., **76**: 559–565. https://doi.org/10.1111/j.1740-0929.2005.00304.x

Tahir, M., Saleh, F., Ohtsuka, A., and Hayahsi, K., 2006. Pectinase plays an important role in stimulating digestibility of a corn-soybean meal diet in broilers. *J. Poult. Sci*., **43**: 323–329. https://doi.org/10.2141/jpsa.43.323

Tahir, M., Saleh, F., Ohtsuka, A., and Hayashi, K., 2008. An effective combination of carbohydrases that enables reduction of dietary protein in broilers: Importance of hemicellulase. *Poult. Sci*., **87**: 713–718. https://doi.org/10.3382/ps.2007-00340

Walters, H.G., 2019. *Evaluating the efficacy of exogenous enzymes on nutrient digestibility and broiler performance.* Doctoral dissertation. https://hdl.handle.net/1969.1/186359

Woyengo, T.A., Slominski, B.A., and Jones, R.O., 2010. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarbohydrase. *Poult. Sci*., **89**: 2221–2229. https://doi.org/10.3382/ps.2010-00832

Yuan, L., Wang, M., Zhang, X., and Wang, Z., 2017. Effects of protease and non-starch polysaccharide enzyme on performance, digestive function, activity and gene expression of endogenous enzyme of broilers. *PLoS One*, **12**: 3. https://doi.org/10.1371/journal.pone.0173941