Pakistan J. Zool., pp 1-7, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231024054103

Impact of Supplementation of Poultry Feed with Locally Characterized Recombinant Thermostable Xylanase on the Growth Performance of Broiler Chicks

Asma Waris[1], Ali Raza Awan[1], Sehrish Firyal[1], Naeem Rashid[2], Abu Seed Hashmi[3], Muhammad Wasim[1], Shagufta Saeed[1] and Muhammad Tayyab[1]*

[1]*Institute of Biochemistry and Biotechnology, Faculty of Biosciences, University of Veterinary and Animal Sciences, Abdul Qadir Jillani (Outfall) Road, Lahore, Punjab, Pakistan.*
[2]*School of Biological Sciences, Faculty of Sciences, University of The Punjab, Quaid e Azam Campus, Lahore, Punjab, Pakistan.*
[3]*Riphah International University, Raiwind Road Campus, Lahore, Pakistan*

Article Information
Received 24 October 2023
Revised 12 February 2024
Accepted 27 February 2024
Available online 23 April 2024
(early access)

Authors' Contribution
AW performed experimental work. MT planned and supervised the study, and guided for manuscript write-up and editing. ASH, SF and SS facilitated the conduction of experiments. MW and ARA helped in data analysis. NR supported in proofreading of manuscript.

Key words
Recombinant thermostable xylanase, XYL_{TN}, *Thermotoga naphthophila*, Poultry trail, weight gain, feed conversion ratio

ABSTRACT

The current study evaluated the efficiency of locally characterized recombinant thermostable xylanase (XYL_{TN}) from *Thermotoga naphthophila* in broiler chicks. The XYL_{TN} was produced using BL21 Codon Plus (DE3) cells having pET-21a containing xylanase gene from *Thermotoga naphthophila* and was used for the supplementation of poultry feed. For the poultry trail, a total of 150 day old broiler chicks were divided into five groups having 30 birds each. Group A served as negative control while groups B, C, and D were experimental groups and fed on a basal diet supplemented with 1000, 1500, and 2000 IU/Kg of locally produced XYL_{TN} respectively, whereas group E served as positive control and was fed on diet supplemented with 1500 IU/Kg of commercially available xylanase. The supplementation of poultry feed with XYL_{TN} revealed, a maximum weight gain of 1681.25g, feed intake of 2810g, and feed conversion ratio of 1.67 when the feed was supplemented with 2000 IU/Kg of XYL_{TN}. Locally produced XYL_{TN} exhibited promising outcomes compared to positive control which is being utilized currently for the supplementation of poultry feed in the industry. The weight gain, feed intake, and feed conversion ratio of 1681.25g, 2810g, and 1.67 for experimental group D were comparable to 1610.38g, 2830g, and 1.75 for positive control. The ability of enzyme to enhance weight gain, feed consumption, and feed conversion ratio in poultry chicks makes it a strong candidate for replacement of its commercial counterpart being imported for the poultry industry, and its domestic production will contribute to the economic availability of this xylanase for the poultry feed industry.

INTRODUCTION

Poultry is one of the most organized and fast-growing agro-based industries in Pakistan and is playing a key role in the fulfilment of the needs for the animal protein of the nation. The high growth rate and feed efficacy are two major considerations of the poultry feed industry. However, the poultry industry is facing a lot of challenges in developing countries and one of the main problems is the expense of feed ingredients which is about 70% of the total production cost (Alagawany and Attia, 2015). The highest production cost of this industry is attributed to the scarcity of cereal grains due to the global increase in demand for these grains, especially corn for ethanol production (Donohue and Cunningham, 2009; O'Nell *at al*., 2012). Thus, aggravated prices of poultry feed enforce the producers to explore alternative, cheaper, and non-conventional feed sources (Ncobela and Chimonyo, 2015).

For poultry feed formulation, protein is one of the main nutrients. Animal protein sources are more expensive whereas plant protein sources are not only cost-effective but also available in abundance, as by-products of the oil seed industry (Beski *et al*., 2015). Hence, the major ingredients of the poultry feed are derived from plant

* Corresponding author: muhammad.tayyab@uvas.edu.pk
0030-9923/2024/0001-0001 $ 9.00/0

sources which are composed of non-starch polysaccharide (NSPs) fibre like cellulose, glucan, mannan, and xylan. However, the limitation in the use of plant-based feed sources is the presence of a high level of NSPs fibres which are not digested by poultry. The presence of dietary NSPs fibre in the poultry feedstuff acts as an anti-nutritional factor and can be accumulated in the form of a gel-like substance in the animal gut which decreases the digestion and absorption of nutrients. Moreover, these SNPs can facilitate intestinal fermentation through modulating intestinal micro-flora that might be harmful to the chicken digestion process (Fathima *et al*., 2022; Choct *et al*., 1996, 1999). Thus, poor digestion and absorption of nutrients lead to the bad performance of birds (Kidd *et al*., 2001; Graham *et al*., 2002; Bedford and Morgan, 1996).

The poultry birds are mono-gastric animals and do not produce endogenous enzymes for the digestion of these fibres. Due to the unavailability of these endogenous enzymes including xylanase, phytase, and cellulase in the animal body, NSPs are being excreted with the manure (Lie and Porress, 2013). To overcome this problem, the diet is supplemented with suitable exogenous enzymes. The usage of exogenous enzymes in poultry feed has been largely studied during the last decades (Bedford and Schulse, 1998). Thus, various types of exogenous enzymes have been recognized for targeting such types of substances, and their effect may be variable depending upon the factors like birds' age, feed quality, and type (Acamovic, 2001; Bedford, 2000). Supplementation of NSPs cleaving enzymes enhances the digestibility of nutrients by decreasing the intestinal viscosity, eliminating the anti-nutritive effect of these NSPs fibre, and also releasing certain bound nutrients which eventually improve the bird performance (Dongare *et al*., 2017). Xylanases are the main enzymes that are involved in the breakdown of xylan by hydrolysing the 1, 4-beta-D-xylosidic linkage between the xylose residues randomly (Mendes *et al*., 2013). The supplementation of feed with xylanase results in the hydrolysis of xylan in feed to simple sugar which improves the nutritive values of the poultry diet that put a positive impact on the growth performance of the poultry birds (Hosseini and Afshar, 2017). Thus, the use of exogenous enzymes like xylanase is one of the key components of the poultry diet for the efficient utilization of feed ingredients and downgrading the production cost (Hahn-Didde and Purdum, 2014).

Thermostability is highly concerned when animal feed is supplemented with exogenous enzymes because, during the pelleting steps, feed is exposed to a very high temperature that denatures mesophilic enzymes (Svihus *et al*., 2005). Thermophilic or hyper-thermophilic microbes can provide thermostable enzymes which can withstand high-temperature conditions without denaturation (Chesson, 1993). The enzymes available in the market are mostly from fungal strains however, the bacterial enzymes have got attention recently because of their specific activity, broad pH range, and higher thermostability (Maki *et al*., 2009). The production cost of these enzymes is high enough, thus there is a need to develop a mechanism for low-cost availability of such thermostable enzymes for industry (Klein *et al*., 2012).

In Pakistan, various groups worked on the production of these enzymes but unfortunately there is no single enzyme available in the market that can be used for the fulfilment of the local industrial demand. The production of the thermostable enzyme by recombinant DNA technology is the most appropriate tool for the economic availability of thermostable enzymes in the market (Kulkami *et al*., 1999; Hough and Danson, 1999).

The current study was designed for the evaluation of the efficacy of locally characterized recombinant thermostable xylanase for the growth of poultry birds and its suitability for the poultry feed industry.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used in the present study were of purified grade and were purchased from Sigma Aldrich (USA) and Merck (Germany).

Production of recombinant xylanase

Recently recombinant thermostable xylanase from *Thermotoga naphthophila* has been characterized. This recombinant xylanase was produced using *E. coli* BL21 CodonPlus cells (DE3) cells having pET-21a containing the xylanase gene from *T. naphthophila*. The microbial culture was maintained on LB agar plates containing ampicillin (100 µg/mL). For the production of recombinant thermostable xylanase under pre-optimized conditions, the LB medium was sterilized in an autoclave (SH-AC-60; SAMHEUNG, Sout Korea). Regarding the production of xylanase, a white colony of overnight grown recombinant BL21 CodonPlus (DE3) cells were diluted to 1% with the fresh LB medium followed by incubation at 37 ºC under shaking conditions until OD reached 0.4. Cells were induced with 0.4 mM of isopropyl β D-1-thiogalactopyranoside and were further incubated under the same conditions. The cells were harvested and re-suspended in 50 mM Phosphate buffer (pH 7) (Tayyab *et al*., 2011).

Cell disruption

The cells were lysed by sonication through the

ultrasonic processor (Sonics, Newtron, USA). The sonicated cells were centrifuged (Z326 K, Hermle Laboratory, Germany) at about 12,000 rpm for 5 min at 4°C (Sabir *at al*., 2017). The supernatant was stored at 4°C for determination of xylanase activity and, for supplementation of the poultry feed.

Xylanase activity assay

Xylanase activity was examined by the dinitrosalisylic acid (DNS) method (Miller, 1959). The total 1000 µL reaction mixture comprised of 50 µL enzyme, 100 µL 1% beech-wood xylan as substrate and 850 µL 50 mM phosphate buffer of pH 7. The enzyme activity assay mixture was incubated for 10 min at 90 °C. The reaction was stopped with the addition of 1mL of DNS reagent followed by boiling for 10 min, cooled for color stabilization and finally the absorbance was recorded at 540 nm. One unit of xylanase activity was defined as the amount of enzyme required to produce one µmol of xylose per min under assay conditions. Xylanase activity units were determined using the standard curve of xylose (Bhalla *et al*., 2015).

Experimental treatment formulation

The soluble portion of sonicated cells was utilized for supplementation of the poultry feed. The feed was formulated in an automated unit at Nizami Feed (Pvt. Ltd), 25 km Sheikhupura, Punjab, Pakistan. The soya bean meal and corn-based feed were prepared for the fulfillment of the nutritional requirements of the poultry chicks, as recommended by the National Research Council (NRC, 1994), and are being used by the poultry industry. The basic composition of feed was the same as being used in commercial feed available for the poultry forms shown in Table I. Five different types of feeds designated as A, B, C, D, and E were formulated, according to the composition given in Table I. Diet A served as negative control and was not supplemented with enzyme whereas diets B, C, and D were supplemented with 1000, 1500, and 2000 IU/Kg of locally produced recombinant xylanase, respectively, while diet E was supplemented with 1500 IU/Kg of commercially available xylanase, Econase XT by AB Vista Animal Nutrition Technology Company (Woodstock, Marlborough, United Kingdom) which served as a positive control. The enzyme concentrations were selected based on literature that indicated the positive response of poultry feed enzymes (Hu *et al*., 2019).

Feeding trials on broiler chicks

The Feeding trials were conducted in the controlled environment at the Dua poultry farms, Niaz Koot, Kala Shah Kaku, tehsil Shahdara, district Lahore, Punjab, Pakistan with the collaboration of Crescent Feeds and Allied Products, Sundar Sharif, Lahore, Punjab, Pakistan. A total of 150-day old broiler chicks of commercial strains were divided into 5 groups each comprises of 3 replicates of 10 birds. Each group was assigned a unique diet as discussed above. The feeding trial was conducted for five weeks (35 days) under controlled conditions and with easy access to feed and water. During the trial, initial, weekly and final body weight, total feed consumed, feed efficacy, feed intake, weight gain, and feed conversion ratio were calculated (Sabir *et al*., 2018).

Table I. Composition of poultry feed.

Feed composition	A	B	C	D	E
Corn	45	45	45	45	45
Rice polish	8.58	8.58	8.58	8.58	8.58
SBM	20	20	20	20	20
SFM	4	4	4	4	4
Oil	3	3	3	3	3
Bone ash	2	2	2	2	2
Canola	12	12	12	12	12
Wheat bran	2	2	2	2	2
Isoleucine	0.01	0.01	0.01	0.01	0.01
Threonine	0.07	0.07	0.07	0.07	0.07
Calcium carbonate	0.23	0.23	0.23	0.23	0.23
L-HCL	0.57	0.57	0.57	0.57	0.57
DCP	1.86	1.86	1.86	1.86	1.86
$NaHCO_3$	0.18	0.18	0.18	0.18	0.18
DLM	0.2	0.2	0.2	0.2	0.2
Premix vitamin	0.3	0.3	0.3	0.3	0.3
Xylanase (IU/Kg feed)	-	1000	1500	2000	1500 (Econase XT)
Sum (kg)	100	100	100	100	100

SBM, soybeans meal; SFM, sunflower meal; Oil, vegetable oil; L-HCl, L-lysine hydrochloride; DCP, Dicalcium phosphate; $NaHCO_3$, sodium bicarbonate; DLM, DL-methionine.

Statistical design

The collected data was analyzed using the SPSS software and one-way ANOVA. The obtained results were represented as the significance of differences between means calculated by the least significant difference test and differences were significant at $P \leq 0.05$ (Sabir *et al*., 2018; Steel *et al*., 1996).

RESULTS AND DISCUSSION

The purpose of the study was to evaluate the impact of supplementation of locally characterized recombinant thermostable xylanase on the growth performance of broiler chicks. The role of xylanase in improving the nutritive values of the poultry diet has been reported previously

(Papadopoulos *et al*., 2022; Bedford, 2000; Bedford and Morgan, 1996; Annison and Choct, 1991). Xylanases are applied as a feed additive to improve the nutritional value of feed resulting in improvement of feed intake, weight gain, and feed conversion of animals (Baker *et al*., 2021; Fisher and Petersson, 2008). The supplementation of poultry feed with locally characterized xylanase showed a growth-enhancing impact in poultry birds and resulted in increased weight gain, feed consumption, and improved FCR. At the end of the first two weeks of trials, a significant impact on bird growth was recorded. The supplementation of feed with 1500 IU/Kg of locally produced xylanase (group C) or 2000 IU/Kg (group D) could enhance the weight of birds from 1050.37 to 1108.32 g or 1270.13 g at the end of the 4th week and from 1410.19 to 1572.31 g or 1681.25 g at the end of 5th week of the trial (Table II) when compared with control. The weight gain data comparison at the end of the trial showed a clear difference in weight gain as compared to the control. The supplementation of feed with locally characterized xylanase with 1000, 1500, and 2000 IU/Kg of feed enhanced the weight gain from 1410.19g for negative control to 1450.16, 1572.31, and 1681.25g for group B, C, and D. The feed supplemented with locally characterized xylanase in group D showed better weight gain 1681.25g as compared to 1610.38g for group E supplemented with 1500 IU/Kg of Econase XT commercially available xylanase. The weight gain of 1681.25g in group D is significantly high as compared to 1610.38g for group E supplemented with commercial Econase XT shown in Table II. These results are in agreement with previous findings regarding improvement in body weight gain in the broilers fed on a corn-soybean-based diet supplemented with xylanase (Rao *et al*., 2021; Hu *et al*., 2019; Olukosi and Bedford, 2019; Dongar *et al*., 2017; Olukosi *et al*., 2007) or wheat-based diet supplemented with xylanase (Pirgozliev *et al*., 2023; Zhang *et al*., 2014; Esmaeilipour *et al*., 2012). However, a greater impact in the wheat-based diet was recorded as compared to the corn-based diet due to the higher amount of soluble NSPs in wheat-based feed (Anwar *et al*., 2023; Nian *et al*., 2011; Hajati, 2010; Mathlouthi *et al*., 2002).

Similarly, the FCR value was improved from 1.88 to 1.67 (group D) which is quite high as compared to 1.75 for Econase XT (group E) which served as a positive control (Table III). The present study revealed the positive effect of locally characterized xylanase supplementation of feed for poultry birds. Thus, FCR was enhanced by the addition of xylanase, which is similar to previous reports (Nian *et al*., 2011; Goa *et al*., 2008). The improved performance of the birds in the present study was might be due to the reduction in the anti-nutritive effect of NSPs due to improved digestion (Kocher *et al*., 2003; Singh *et al*., 2012; Stefanello *et al*., 2015; Vandeplas *et al*., 2010; Malathi and Devegowda, 2001).

CONCLUSION

The locally characterized recombinant thermostable xylanase has strong potential for NSP_s digestion and for enhancement of weight gain, feed consumption and improved FCR value in broiler chicks. The weight gain data clearly showed a significant impact of locally characterized xylanase as compared to Econase XT, xylanase being utilized currently in poultry feed. Domestic production of locally characterized xylanase will save the huge foreign exchange for import of this enzyme. The local production of this enzyme will result in the economic availability of xylanase that can replace the imported counterpart being utilized currently in the poultry feed industry.

Table II. Effect of xylanase on weight gain data of poultry trials.

Groups	Week 1 P= (0.000***)	Week 2 P= (0.000***)	Week 3 P= (0.000***)	Week 4 P= (0.000***)	Week 5 P= (0.000***)
A (Negative control)	160.87±0.81	407.73±0.59	741.61±0.24	1050.37±0.61	1410.19±0.42
B (1000 IU/Kg)	166.67±0.59	416.89±0.45	757.16±0.57	1087.15±0.37	1450.16±0.64
C (1500 IU/Kg)	177.60±0.51	427.25±0.89	766.51±0.42	1108.32±0.27	1572.31±0.33
D (2000 IU/Kg)	181.53±0.41	477.92±0.80	799.22±0.74	1270.13±0.71	1681.25±0.56
E (1500 IU/Kg) (Econase XT)	180.12±0.33	431.83±0.45	784.72±0.47	1140.17±0.29	1610.38±0.89

Table III. Efficacy of xylanase in weight gain, feed intake and feed conversion ratio.

Groups	A negative control	B 1000 IU/Kg	C 1500 IU/Kg	D 2000 IU/Kg	E 1500 IU/Kg (Econase XT)
Average feed intake (g)	2650	2700	2770	2810	2830
Overall weight gain (g)	1410±0.42	1472±0.33	1572±0.56	1681±0.56	1610 ±0.89
FCR	1.88	1.84	1.76	1.67	1.75

ACKNOWLEDGEMENT

The authors are thankful to Nizami Feed (Pvt. Ltd), Sheikhupura, Punjab, Pakistan for all their support in the conduction of trails.

Funding

The study received no external funding.

IRB approval

This study was approved by the Advanced Studies Research Board of University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan

Ethical statement

The experiments were conducted with the prior approval of Animal Ethics Committee, University of Veterinary and Animal Sciences Lahore, and with Pakistan code for the care and the use of animals for scientific purposes.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Acamovic, T., 2001. Commercial application of enzyme technology for poultry production. *Worlds Poult. Sci. J.*, **57**: 251-263. https://doi.org/10.1079/WPS20010016

Alagawany, M. and Attia, A.I., 2015. Effects of feeding sugar beet pulp and avizyme supplementation on performance, egg quality, nutrient digestion and nitrogen balance of laying Japanese quail. *Avian Biol. Res*., **8**: 79-88. https://doi.org/10.3184/175815515X14274754281188

Annison, G. and Choct., 1991. Anti-nutritive activities of cereal non-starch polysaccharide in broiler diets and strategies minimizing their effects. *Worlds Poult. Sci. J.*, **47**: 232-247. https://doi.org/10.1079/WPS19910019

Anwar, U., Riaz, M., Khalid, M.F., Mustafa, R., Farooq, U., Ashraf, M., Munir, H., Auon, M, Hussain, M., Hussain, M., Ayaz, Chisti, M.F., Bilal, M.Q., Rehman, A.U. and Rahman, M.A.U., 2023. Impact of exogenous xylanase and phytase, individually or in combination, on performance, digesta viscosity and carcass characteristics in broiler birds fed wheat-based diets. *Animals* (Basel), **13**: 278. https://doi.org/10.3390/ani13020278

Baker, J.T., Duarte, M.E., Holanda, D.M. and Kim, S.W., 2021. Friend or Foe? Impacts of dietary xylans, xylooligosaccharides, and xylanases on intestinal health and growth performance of monogastric animals. *Animals*, **11**: 609. https://doi.org/10.3390/ani11030609

Bedford, M., 2000. Exogenous enzymes in monogastric nutrition, their current value and future benefits. *Annls Feed Sci. Technol*., **86**: 1-13. https://doi.org/10.1016/S0377-8401(00)00155-3

Bedford, M.R. and Morgan, A., 1996. The use of enzymes in poultry diets. *Worlds Poult. Sci. J*., **52**: 61-68. https://doi.org/10.1079/WPS19960007

Bedford, M.R. and Schulse, H., 1998. Exogenous enzyme for pigs and poultry. *Nutr. Res. Rev*., **11**: 91-114. https://doi.org/10.1079/NRR19980007

Beski, S.S.M., Swick, R.A. and Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. *Anim. Nutr*., **1**: 47-53. https://doi.org/10.1016/j.aninu.2015.05.005

Bhalla, A., Bischoff, K.M. and Sani, R.K., 2015. Highly thermostable xylanase production from a thermophilic *Geobacillus* sp. strain WSUCF1 utilizing lignocellulosic biomass. Front. *Bioeng. Biotechnol*., **3**: 84. https://doi.org/10.3389/fbioe.2015.00084

Chesson, A., 1993. Feed enzymes. *Anim. Feed Sci. Technol*., **45**: 65-79. https://doi.org/10.1016/0377-8401(93)90072-R

Choct, M., Hughes, R.J. and Bedford, M.R., 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine and ileal and caecal volatile fatty acid production in chickens fed wheat. *Br. Poult. Sci*., **40**: 419-422. https://doi.org/10.1080/00071669987548

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J. and Annison, G., 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *Br. Poult. Sci*., **37**: 609-621. https://doi.org/10.1080/00071669608417891

Dongare, N.A., Deshmukh, A.D., Dhok, A.P, Lende, S.R. and Taksande, P.E., 2017. Supplementation of protease and xylanase enzymes in broiler diet with varying energy and protein levels. *Int. J. Curr. Microbiol. appl. Sci*., **6**: 1715-1720. https://doi.org/10.20546/ijcmas.2017.611.207

Donohue, M. and Cunningham, D.L., 2009. Effects of grain and oilseed prices on the costs of US poultry production**.** *J. appl. Poult. Res*., **18**: 325-337. https://doi.org/10.3382/japr.2008-00134

Esmaeilipour, O., Moravej, M., Shivazad, M., Rezaian, S., Aminzadeh and Krimpen, V.M.M., 2012. Effects of diet acidification and xylanase supplementation

on performance, nutrient digestibility, duodenal histology and gut microflora of broilers fed wheat based diet. *Br. Poult. Sci*., **53**: 235-244. https://doi.org/10.1080/00071668.2012.681771

Fathima, S., Shanmugasundaram, R.A., Dams, D. and Selvaraj, R.K., 2022. Gastrointestinal microbiota and their manipulation for improved growth and performance in chickens. *Foods*, **11**: 1401. https://doi.org/10.3390/foods11101401

Fisher, M. and Petersson, D., 2008. *Xylanases for animal feed, Assigne: Novozymes.* International patent. WO/2008/037757.

Goa, F., Jiang, Y., Zhuo, G.H. and Han, Z.K., 2008. The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut microflora in broilers fed on wheat-based diets. *Anim. Feed Sci. Technol*., **142**: 173-184. https://doi.org/10.1016/j.anifeedsci.2007.07.008

Graham, K., Kerley, k., Firman, M.S. and Allee, G.L., 2002. The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. *Poult. Sci*., **81**: 1014-1019. https://doi.org/10.1093/ps/81.7.1014

Hahn-Didde, D. and Purdum, S.E., 2014. The effects of an enzyme complex in moderate and low nutrient-dense diets with dried distillers grains with soluble in laying hens. *J. appl. Poult. Res*., **23**: 23-33. https://doi.org/10.3382/japr.2013-00764

Hajati, H., 2010. Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. *Am. J. Anim. Vet. Sci*., **5**: 221-227. https://doi.org/10.3844/ajavsp.2010.221.227

Hosseini, S.M. and Afshar, M., 2017. Effects of feed form and xylanase supplementation on performance and ileal nutrients digestibility of heat-stressed broilers fed wheat-soybean diet. *J. appl. Poult. Res*., **45**: 550-556. https://doi.org/10.1080/09712119.2016.1224765

Hough, D. and Danson, M., 1999. Extremozymes. *Curr. Opin. Chem. Boil*., **3**: 39-46. https://doi.org/10.1016/S1367-5931(99)80008-8

Hu, H., Dai, S., Wen, A. and Bai, X., 2019. Efficient expression of xylanase by codon optimization and Its effects on the growth performance and carcass characteristics of broiler. *Animal*, **9:** 65. https://doi.org/10.3390/ani9020065

Kidd, M.T., Morgan, G.W. and Zumwalt, C.D., 2001. Galactosidase enzyme supplementation to corn and soybean meal broiler diets. *J. appl. Poult. Res*., **10**: 186-193. https://doi.org/10.1093/japr/10.2.186

Klein, M.D., Oleskowicz, P.P., Simmons, B.A. and Blanch, H.W., 2012. The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnol. Bioeng*., **109**: 1083-1087. https://doi.org/10.1002/bit.24370

Kocher, A., Choct, M., Ross, G., Broz, J. and Chung, T.K., 2003. Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal-based diets in broilers. *J. appl. Poult. Res*., **12**: 275-283. https://doi.org/10.1093/japr/12.3.275

Kulkami, N., Shendye, A. and Rao, M., 1999. Molecular and biotechnological aspects of xylanases. *FEMS Microbiol. Rev.*, **23**: 411-456. https://doi.org/10.1111/j.1574-6976.1999.tb00407.x

Lie, X.G. and Porress, J.M., 2003. Phytase enzymology, application and biotrchnology. *Biotechnol. Lett*., **25**: 1787-1794. https://doi.org/10.1023/A:1026224101580

Maki, M., Leung, K.T. and Qin, W., 2009. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int. J. biol. Sci*., **5**: 500-516. https://doi.org/10.7150/ijbs.5.500

Malathi, V. and Devegowda, G., 2001. *In vitro* evaluation of nonstarch polysaccharide digestibility of feed ingredients by enzymes. *Poult. Sci*., **80**: 302-305. https://doi.org/10.1093/ps/80.3.302

Mathlouthi, N., Mallet, S., Saulnier, L., Quemener, B. and Larbier, M., 2002. Effect of xylanase and glucanase addition on performance, nutrient digestibility and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. *Anim. Res*., **51**: 395-406. https://doi.org/10.1051/animres:2002034

Mendes, A.R., Ribeiro, T., Correia, B.A., Bule, P., Maçãs, B., Falcão, L., Freire, J.P.B., Ferreira, L.M.A., Fontes, C.M.G.A. and Lordelo, M.M., 2013. Low doses of exogenous xylanase improve the nutritive value of triticale-based diets for broilers. *J. appl. Poult. Res*., **22**: 92-99. https://doi.org/10.3382/japr.2012-00610

Miller, G.L., 1959. Use of dinitrosalicylic acid as reagent for the determination of reducing sugars. *Anal. Chem.*, **31**: 426-428. https://doi.org/10.1021/ac60147a030

National Research Council, 1994. *Nutrient requirements of poultry*. Ninth revised edition, *1994*. The National Academies Press, Washington DC.

Ncobela, C.N. and Chimonyo, M., 2015. Potential of using non-conventional animal protein sources for sustainable intensification of scavenging

village chickens: A review. *Anim. Feed Sci. Technol*., **208**: 1-11. https://doi.org/10.1016/j.anifeedsci.2015.07.005

Nian, F., Guo, Y.M., Ru, Y.J., Péron, A. and Li, F.D., 2011. Effect of xylanase supplementation on the net energy for production, performance and gut microflora of broilers fed Corn/soy-based diet. *J. Anim. Sci*., **24**: 1282-1287. https://doi.org/10.5713/ajas.2011.10441

O'Nell, H.V.M., Mathis, G.B.S., Lumpkins and Bedford, M.R., 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. *Poult. Sci.,* **91**: 1356-1360. https://doi.org/10.3382/ps.2011-01867

Olukosi, A. and Bedford, M.A., 2019. Comparative effects of wheat varieties and xylanase supplementation on growth performance, nutrient utilization, net energy, and whole-body energy and nutrient partitioning in broilers at different ages. *Poult. Sci*., **98**: 2179-2188. https://doi.org/10.3382/ps/pey582

Olukosi, O., Bedford, A. and Adeola, M.R., 2007. Xylanase in diets for growing pigs and broiler chicks. *Can. J. Anim. Sci*., **87**: 227-235. https://doi.org/10.4141/CJAS06005

Papadopoulos, G.A., Lioliopoulou, S., Ordoudi, S.A, Giannenas, I., Hoeck, V., Morisset, D., Arsenos, G., Fortomaris, P. and Mantzouridou, F.T., 2022. Xylanase supplementation in wheat-based diets of laying hens affects the egg yolk color, carotenoid and fatty acid profiles. *Foods*, **11**: 2209. https://doi.org/10.3390/foods11152209

Pirgozliev, V.R., Mansbridge, S.C., Whiting, I.M., Abdulla, J.M., Rose, S.P., Kljak, K., Johnson, A., Drijfhout, F. and Atanasov, A.G., 2003. The Benefits of exogenous xylanase in wheat–soya based broiler chicken diets, consisting of different soluble non-starch polysaccharides content. *Poultry*, **2**: 123-133. https://doi.org/10.3390/poultry2020012

Rao, S.V.R., Raju, M.V.L., Nagalakshmi, D., Prakash, B. and Paul, S.S., 2021. Effect of supplementation of graded concentrations of xylanase and a-amylase on performance, slaughter variables, and energy digestibility in broiler chickens fed corn-soya bean meal–based diet. *Appl. Poult. Res*., **30**: 100139. https://doi.org/10.1016/j.japr.2021.100139

Sabir, F., Tayyab, M., Awan, A. R., Muneer, B., Hashmi, A.S., Wasim, M. and Firyal, S., 2018. Biological evaluation of locally produced recombinant Phytase in broiler chicks. *J. Anim. Plant Sci*., **28:** 946-950.

Sabir, F., Tayyab, M., Muneer, B., Hashmi, A.S., Awan, A.R., Rashid, N., Wasim, M. and Firyal, S., 2017. Characterization of recombinant thermostable phytase from *Thermotoga naphthophila*: A step for the fulfilment of domestic requirement of phytase in Pakistan. *Pakistan J. Zool*., **49**: 1945-1951. https://doi.org/10.17582/journal.pjz/2017.49.6.1945.1951

Singh, A., Neill, H.M.O., Ghosh, T.K., Bedford, M.R. and Haldar, S., 2012. Effects of xylanase supplementation on performance, total volatile fatty acids and selected bacterial population in caeca, metabolic indices and peptide YY concentrations in serum of broiler chickens fed energy restricted maize soy bean based diets. *Anim. Feed Sci. Technol*., **177**: 194-203. https://doi.org/10.1016/j.anifeedsci.2012.08.005

Steel, R.G.D., Torrie, J.H. and Dicky, D.A., 1996. *Principles and procedures of statistics: A biometrical approach*. 3rd Edition Published by McGraw Hill, College, USA.

Stefanello, C., Vieira, S.L., Santiago, G.O., Kindlein, L., Sorbara, J.O.B. and Cowieson, A.J., 2015. Starch digestibility, energy utilization and growth performance of broilers fed corn-soybean basal diets supplemented with enzymes. *Poult. Sci*., **94**: 2472-2479. https://doi.org/10.3382/ps/pev244

Svihus, B., Uhlen, A.K. and Harstad, O.M., 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review *Anim. Feed Sci. Technol*., **122**: 303-320. https://doi.org/10.1016/j.anifeedsci.2005.02.025

Tayyab, M., Rashid, N. and Akhtar, M., 2011. Isolation and identification of lipase producing thermosphilic *Geobacillus* sp. SBS-4S: Cloning and characterization of lipase. *J. Biosci. Bioeng*., **111**: 272-278. https://doi.org/10.1016/j.jbiosc.2010.11.015

Vandeplas, S., Dauphin, R.D., Thonart, P., Théwis, A. and Beckers, Y., 2010. Effect of the bacterial or fungal origin of exogenous xylanases supplemented to a wheat-based diet on performance of broiler chickens and nutrient digestibility of the diet. *Can. J. Anim. Sci*., **90**: 221-228. https://doi.org/10.4141/CJAS09067

Zhang, L., Xu, J., Lei, L., Jiang, Y., F. Gao, F. and Zhou, G.H., 2014. Effects of xylanase supplementation on growth performance, nutrient digestibility and non-starch polysaccharide degradation in different sections of the gastrointestinal tract of broilers fed wheat-based diets. *Asian-Aust. J. Anim. Sci*., **27**: 855-861. https://doi.org/10.5713/ajas.2014.14006