

Research Article



Enhancing Broiler Chick Growth with Locally Produced Thermostable Cellulase in Fibrous Diets

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Abstract | Cellulase has been utilized in poultry plant-based diets to enhance nutritional value by neutralizing anti-nutritional factors. This study investigated the effects of a recombinant thermostable cellulase from *Thermotoga naphthophila* (CELTN) on the growth performance of broiler chicks when added to corn-based feed. The thermostable cellulase was produced using BL21 CodonPlus cells with the pET28a-Cellu vector under optimized conditions. A total of 150 one-day-old, unsexed broiler chicks were divided into five treatment groups, each further subdivided into three replicates of 10 chicks with an initial average weight of 39 grams. Group A served as the negative control with a non-enzyme supplemented diet, while Group E was the positive control, receiving a diet supplemented with 500 IU of commercial neutral cellulase. Groups B, C, and D received diets supplemented with locally produced recombinant thermostable cellulase at concentrations of 500, 750, and 1000 IU/kg, respectively. The results, analyzed using ANOVA and Duncan's Multiple Range Test, showed significant improvements ($P < 0.05$) in weight gain, feed intake, and feed efficiency ratio for enzyme-treated groups, particularly from the 3rd to 7th week of the trial. Group D, with 1000 IU of the local cellulase, demonstrated the most significant effects, with a weight gain of 3460.11 grams, feed intake of 6332.40 grams, and a feed conversion ratio (FCR) of 1.83. These results indicate that the inclusion of locally produced thermostable cellulase up to 1000 IU/kg in broiler feed significantly enhances growth performance ($P < 0.05$). This pilot study suggests that CELTN is a viable candidate for the poultry feed industry and could improve economic efficiency by reducing reliance on imported enzymes.

Keywords | CELTN; Feed Supplementation; Feed Intake and Feed Efficiency Ratio; *Thermotoga naphthophila*; Poultry Trial; Weight Gain

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INTRODUCTION

Poultry farming is a major sector in global agribusiness recognized for its profitability and growth. Since the 1980s, advancements in technology, particularly the use of food additives, have significantly enhanced the nutritional quality of poultry feed (Rao, 2020). In the current era, increasing population and their changing habit of diet towards animal protein put more pressure on demand. Now there is a need to improve the practices in the livestock

sector to meet production demand and improve the health of animals (Rehman et al., 2017). Feed additives are products or combinations of different products supplemented in the basic feed of animals for improving the animals' performance and health. Feed additives, which are generally added to animal feed, are of two types: nutritive feed additives such as amino acids, vitamins and minerals etc., and non-nutritive feed additives such as antibiotics, immunoglobulins, hormones, probiotics and enzymes, etc., (Philipps-Wiemann, 2018).

In Pakistan, like other developing countries, the poultry industry is facing some challenges due to high cost of feed (Sarwat et al., 2022). Currently, 75% of the total cost of broiler chick depends on the feed (| Ministry of Finance | Government of Pakistan | n.d.), The high price of the poultry diet forced farmers to use alternative cheap conventional feedstuffs commonly based on corn and soybean meal. Recently, the use of plant-based feed as a source of nutrient and energy received great attention all around the developing countries (Sarwat et al., 2022; Świątkiewicz, Książak and Hanczakowska, 2018). A high proportion of non-soluble polysaccharide (NSP) in plant-based feed acts as anti-nutritional factor and adversely affects the health of animals at commercial scale (İncili et al., 2020; Reda et al., 2020; Alfaia et al., 2021).

The nutritional value of corn or soybean-based diet is limited for mono-gastric animals due to high proportion of undigestible fiber contents. However, this obstacle could be resolved by the addition of exogenous enzymes in poultry diet to drive optimal nutrients from complex feed metrics (Marchiori et al., 2022). Studies confirmed that the addition of exogenous enzymes individually or in combination improves bird performance via enhancing the digestibility capacity (Lu et al., 2013). Exogenous enzymes improve the nutritional value of plant-based feed by breaking down the specific bond in complex structure of biomolecules. In the last few years, enzymes have been used largely in the animal feed industry to check the efficacy of new products (Karunaratne et al., 2021). Enzymes used in the animal feed industry break down the fibrous and cellulose contents which are chiefly ubiquitous in cereals and plant-based feed (Menezes-Blackburn and Greiner, 2015).

Over the last few years, various enzymes such as cellulase (β -glucanases), xylanases, phytases, proteases, lipases, and galactosidase have been potentially used in poultry feed industry to improve the animal performance via maintain the available nutrient in poultry diet (Bedford and Apajalahti 2022a). Li investigation with his colleague in 2020 also proved that cellulase supplementation in poultry feed enhanced the digestibility of dominant cellulosic portion especially present in corn and soybean-based feed (Li et al. 2020).

Cellulase a complex form of enzymes which are secreted by the broad range of microorganisms such as bacteria, fungi and actinomycetes belong to hydrolase family and specifically break down the complex structure of cellulose into simpler glucose components (Mousavi et al., 2022).

Researchers also reported that the supplementation of cellulase in poultry feed significantly enhanced the weight gain and feed intake ratio via proper catalysis of cellulosic

portion in corn or soybean-based feed (Velázquez-De Lucio et al., 2021).

In the poultry feed industry, pelleting is a crucial step that agglomerates the ingredient mixture, increases nutrient digestibility, reduces feed wastage, and minimizes microbial contamination through the application of high heat, pressure, and humidity. Among these factors, temperature—specifically around 90 °C—has a significant impact, as it can irreversibly denature enzymatic components added to the feed mix (Teixeira Netto et al., 2019). This challenge drives scientists to develop thermostable enzymes, recognizing the critical importance of the pelleting process in poultry feed production. On an industrial scale, thermostable cellulase is considered as the ideal candidate to withstand the harsh feed pelatinization condition (Zamost et al., 1991; Kuhad et al., 2011; Sabir et al., 2018; Vasudevan et al., 2019). This study aims to evaluate the effects of locally produced thermostable cellulase (CELTN) on the growth performance of broiler chickens.

MATERIALS AND METHODS

The experimental design and procedure were reviewed and approved by the Ethical Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan.

RECOMBINANT CELLULASE PRODUCTION

BL21 CodonPlus cells transformed with a pET28a expression vector harboring the hyperthermophile *T. naphthophila* strain cellulase gene from the Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, were utilized in this study (Khalid et al., 2019). All production steps were carried out under pre-optimized conditions for maximal enzyme production as reported by Khalid et al. (2019). The overnight grown cells were diluted with final concentration of 1% fresh LB broth and incubated again at 37 °C in a shaker incubator until the OD values reached 0.6. Then the cells were incubated again at 25 °C for 22 h after IPTG induction with a final concentration of 0.5 mM. In the final steps cells were re-suspended in 50 mM buffer pH 7 after harvesting via centrifugation (Russell and Sambrook, 2001).

CELL DISRUPTION AND ENZYME ACTIVITY

Cellulase activity from cleared supernatant was examined after the centrifugation of sonicated material which is followed for lysis of recombinant harvested cells. The enzymatic activity assay was performed according to Zhang et al. (2009). The reaction mixture was prepared by taking the 1.25 μ L cobalt chloride, 250 μ L of Carboxymethyl cellulose, 50 μ L of enzyme and 198.75 μ L 50 mM sodium acetate buffer of pH 4.8 in falcon. The reaction mixture was incubated at 90 °C for 30 minutes and then 1 mL volume

Table 1: The percentage composition of feed ingredients in different treatment groups

Feed Ingredients	Diet A (%)	Diet B (%)	Diet C (%)	Diet D (%)	Diet E (%)
Corn	60.2	60.2	60.2	60.2	60.2
Soybean meal	30.0	30.0	30.0	30.0	30.0
Corn Gluten Meal	2.5	2.5	2.5	2.5	2.5
Soybean Oil	2.8	2.8	2.8	2.8	2.8
Limestone	1.2	1.2	1.2	1.2	1.2
Dicalcium phosphate	1.7	1.7	1.7	1.7	1.7
Vitamin-mineral Premix	1.0	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.3
L-Lysine	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.15	0.15	0.15	0.15	0.15
Cellulase	-	500 IU	750 IU	1000 IU	500 IU (Commercial, Neutral Cellulase)
Total	100	100	100	100	100

of DNS was added and boiled at 100 °C in the water bath (D- 91126, Memmert, Germany) for 10 minutes and took absorbance at 540 nm. The experiment was performed in triplicate (Khalid et al., 2019). The protein concentration was estimated by Bradford method using bovine serum albumin as standard (Bradford, 1976).

FEED FORMULATION FOR TRIALS

The poultry feed based on corn meal was prepared in an automated unit available at Hi-Tech Feed (Pvt.) Ltd (23rd K.M. Raiwind Road). The percentage composition of the feed components was the same as being used in control shed for the birds and five experimental groups (A to E) that only be varied in cellulase units. Diet A was taken as negative control and lack cellulase while diet E was supplemented with the commercially available Neutral cellulase (500 IU/Kg) and acted as a positive control (Table 1). The diet B, C and D groups were supplemented with different concentrations of locally produced cellulase i.e 500 IU/Kg, 750 IU/Kg and 1000 IU/Kg of feed, respectively (Table 1).

PROXIMATE ANALYSIS OF FEED

The proximate analysis of poultry feed was carried out by using standard methods for dry matter (DM), crude protein (CP), total ash (TA) as reported by Samar et al. (2023). While, for metabolized energy, amino acids (Methionine and cysteine) and minerals (Calcium and Phosphorus) methods of Ravindran et al. (2014) were followed with few modifications.

POULTRY TRIAL EXPERIMENT

The feeding trails were conducted in an environmentally controlled house (Aliyas protein farm, Lahore, Pakistan). One day old broiler chicks (average weight 39 g per chick),

totaling 150, were used in a 7-week trials. The birds were divided into five groups, and each group was further divided into three subgroups containing 10 birds each. Group A served as the negative control, while Group E served as the positive control, with each group fed ration formulation diets A and E, respectively. Diets B, C, and D were prepared by Hi-Tech Feed (Pvt.) Ltd. according to different enzyme units and fed to Groups B, C, and D, respectively, for 49 days. All birds were housed in 15 poultry pens under continuous light and a bell feeder (Ji et al., 2008; Adeola and Cowieson, 2011).

STATISTICAL ANALYSIS

The bird’s body weight was recorded regularly every week to check the performance variable of weight gain, feed intake and feed conversion ratio during trial. Duncan’s multiple range tests and one way ANOVA were applied on collected trail data to find out the significant differences of means of all groups and variables of trail data (George-Oka and Mike-Anosi, 2012; Saminathan and Sriman-Narayanan, 2015). The LSD (least significant difference) test was also performed to find the significance of means at P < 0.05 (Heinisch, 1962).

RESULTS AND DISCUSSION

The proximate analysis of the feed was done, and the detected amount of metabolized energy, dry matter, crude protein, total ash, two amino acids (methionine and cysteine) and two minerals such as calcium and phosphorus were done and mentioned in Table 2. The soyabean based poultry feed is good in protein contents, minerals, and amino acids compared to Whole Prosopis pods meal (Al-Harathi et al., 2019). Similarly different types of Soy bean meal

Table 2: A nutrient composition of poultry feed

Nutritional Ingredients	Value
Metabolizable Energy (ME)	3122 kcal/kg
Dry Matter (DM)	89%
Crude Protein (CP)	20.72 %
Total Ash (TA)	6.57%
Methionine + Cysteine	0.65 mg/kg
Calcium	0.9 mg/kg
Phosphorus	0.6 mg/kg

Table 3: Efficacy of CELTN supplementation on weight gain, feed intake and feed efficiency ratio

Groups	A (Negative Control)	B (500 IU/Kg CELTN)	C (750 IU/Kg CELTN)	D (1000 IU/Kg CELTN)	E (500 IU/Kg neutral cellulase)
Overall, Weight gain (g/Chick)	3145.01 ± 13.18	3240.31 ± 18.05	3357.01 ± 13.03	3460.11 ± 19.16	3210.11 ± 15.71
Average Feed Intake (g/Chick)	6217.40 ± 97.72	6250.40 ± 83.86	6260.20 ± 93.72	6332.40 ± 67.99	6311.80 ± 69.22
FCR	1.976	1.928	1.864	1.830	1.966

(SBM) varied in nutritional components as US Soybean meal (SBM) has higher crude protein (47.3%), better digestible protein, and amino acid content compared to SBM in our study that had the lowest protein and amino acid digestibility, while US SBM exhibited superior overall nutritive value (Ravindran et al., 2014).

The cellulase-treated feed groups showed significant performance of broiler chick from 1st week to 7th week as compared to the negative control group. The feed group supplemented with 500 IU/kg showed an enhancing effect on the growth performance of birds as compared to the negative control while results of Group C fed with diet C (750 IU/kg) were found to be comparable with group E having commercial enzyme. However, the best performance results were obtained in the case of group D, fed with 1000 IU/Kg CELTN (Fig. 1). It is reported by In-borr et al. (1999) that feeding pigs diets treated with cellulase or xylanase increased stomach and ileal xylanase and β-glucanase activities, impacting nutrient digestion that is directly related to growth of the animal.

The feed supplemented with CELTN (1000 IU/kg) showed more significant results on growth and weight gain performance of broiler among other feed groups containing 500 and 700 IU/kg after the 7th week of the trial. The results clearly demonstrated that increased units of locally produced CELTN lead to significant weight gain as compared to group E supplemented with commercially produced enzyme. It was also concluded that an increase in enzyme units had a more significant result on broiler birds.

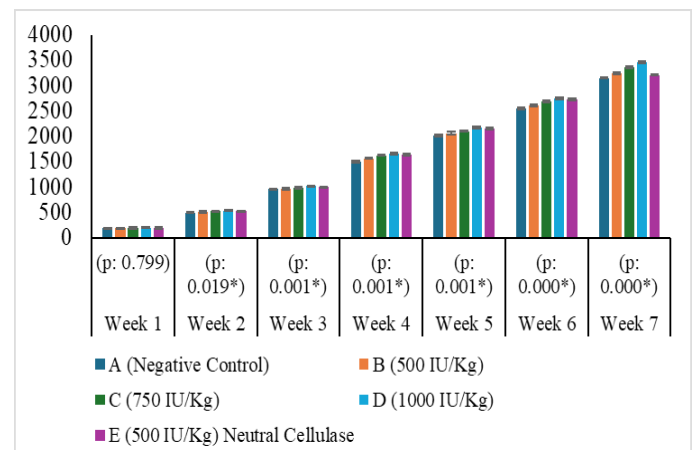


Figure 1: Growth Performance (weight gain, g) Data of Poultry Birds supplemented various levels of CELTN. *: p-value less than 0.05.

These results agree with previous reports of Ahmad et al. (2014); Dalólio et al. (2016); Nunes et al. (2015). They also reported that the digestibility capacity of a low-quality non-starch polysaccharide diet improved with the supplementation of exogenous cellulase. They also observed that the digestibility capacity of non-starch polysaccharides increased by using exogenous cellulase. On the other hand some studies (Saleh et al., 2018; Choct et al., 1995; Guerreiro et al., 2008; ; Leite et al., 2011; Dalólio et al., 2016; Ponte et al., 2004) were found to be contradictory with our data presented the non-significant effect of addition of exogenous cellulase on relative weight gain of broiler.

This study also found the significant result of diet D (1000

AUTHORS CONTRIBUTION

Aisha Khalid did experimental work, Muhammad Tayyab was supervisor and planned research work, Roheela Yasmeen did final drafting of the final manuscript.

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IU/kg) on average feed intake value (Table 3). Similarly, the feed consumption ratio of group D was found to be lowered (1.850) as compared to the other groups, indicating its overall positive effect on bird health. The results of (Makhdum et al., 2013; Ahmad et al., 2014; Nunes et al., 2015;) agree with our finding. They also observed that the cellulase treated group had significant effect on FCR value, while (Yonemochi et al., 2003; Cowieson and Adeola 2005; Olukosi et al., 2007; Zhao et al., 2008; Kaczmarek et al., 2009) results are in-agreement with our data, reporting the non-significant effect of cellulase on feed conversion ratio. The locally produced cellulase also enhanced the feed intake value of the broiler as compared to the negative group, which was not treated with any enzyme. Among the different groups, group D determined the highest value of 6332.40 g, showing its more significant result. These results are in accordance with Zulkarnain et al. (2017) indicating the encouraging result of exogenous cellulase supplementation in chick feed. On the other hand, Yu and Chung, (2004); Gao et al. (2007); del Alamo et al. (2008); Lu et al. (2013) reported the non-significant effect of enzyme on feed intake value.

CONCLUSION

Locally produced thermostable CELTN supplementation in feed significantly improved the growth performance of broiler chicks and can be considered an important industrial candidate to meet the requirements of Pakistan's poultry feed industry at the commercial level.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

NOVELTY STATEMENT

This study is novel as it evaluates the effects of a recombinant thermostable cellulase (CELTN) from *Thermotoga naphthophila* on broiler growth performance when added to a corn-based feed. The use of locally produced CELTN at varying concentrations shows significant improvements in growth metrics, presenting a cost-effective alternative to imported enzymes for the poultry industry.

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