BIOLOGICAL EVALUATION OF LOCALLY CHARACTERIZED RECOMBINANT THERMOSTABLE α-AMYLASE IN POULTRY BIRDS

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ABSTRACT

Current study deals with the biological evaluation of locally characterized recombinant thermostable α-amylase in poultry broiler chicks. The enzyme was produced under pre-optimized conditions using *E.coli* BL21CodonPlus (DE3) cells transformed with pET21a containing α-amylase gene from *Thermotoga naphthophila.* The enzyme was utilized for the supplementation of poultry feed. Regarding poultry trials, 150 day-old broiler chicks were divided randomly into 5 groups with 30 birds in each group. Group A served as negative control and was fed on standard feed without supplementation. Group B, C and D were fed on feed supplemented with 200, 400 and 600 IU/kg of locally produced recombinant thermostable α-amylase while group E served as positive control and was fed on feed supplemented with commercially available α-amylase. The birds were housed under controlled conditions for a period of 7 weeks. Supplementation of poultry feed with 600 IU/kg recombinant α-amylase resulted in significant enhancement of weight gain from 596g (Group A) to 694g (Group D), and improved FCR value from 2.17 (Group A) to 1.95 (Group D) at the end of 7th week. Group D showed promising weight gain and improved FCR value of 694g and 1.95 as compared to 666g and 2.09 for group E supplemented with commercially available α-amylase. Locally produced thermostable recombinant α-amylase showed committed results and proves its potential for its utilization in poultry feed industry. Its domestic production at large scale will save huge foreign exchange for the import of this enzyme.

Index terms- Recombinant α-amylase, *Thermotoga naphthophila*, Thermostable, Feed industry, Feed Conversion Ratio, Weight gain

1. INTRODUCTION

Poultry industry is one the most dynamic segment of agriculture in Pakistan with significant contribution in Pakistan's GDP (1.4%). Poultry has become the 2^{nd} largest industry in Pakistan and 11^{th} largest producer in the world which is responsible for providing employment to 1.5 million people (Aslam et al., 2020). Poultry industry of Pakistan is responsible in bridging the gap between supply and demand for population. This industry is facing problem due to high cost of feed and this is the major issue for high rate of poultry meat in market (Samad et al., 2022). The poultry sector has started exploring alternate sources for cheaper availability of feed for broilers (Deek et al., 2020).

Primary components of plant based poultry feed are from soyabean, corn, grains and wheat (Jha and Mishra, 2021; Anjum et al., 2014). Plant based poultry feed have high fibre content which includes Non-starch polysaccharide (NSPs) that is not digested by the poultry bird due to unavailability of enzymes in monogastric animals (Alagawany et al., 2018; Kim et al., 2022). Secondly, presence of NSP increases the viscosity in digestive tract that is another factor for improper digestion. To overcome this issue, poultry feed is being supplemented with enzymes resulting in the conversion of complex, non-digestible molecules to simple digestible units which can be utilized as energy source (Tiquia and Tam, 2002; Castro et al., 2020).

Enzymes are helpful in breakdown of complex non-starch polysaccharides and provide additional source of energy for bird growth (Algawany et al., 2018). Various enzymes like phytase, protease, amylase, cellulase and xylanase are important for the supplementation of the poultry feed for proper digestion and enhanced growth rate (Marchiori et al., 2022; Sabir et al., 2018, Khalid et al., 2019; Waris et al., 2024). Digestion of starch in poultry happen when pancreatic amylase encounters ingested starch polymers (Cowieson et al., 2019). Earlier studies have shown that starch digestion is not completed in duodenum of young chicks due to inadequate amount of amylase so, there is a need of exogenous enzyme to enhance the performance in birds (Yuan et al., 2017).

α-amylase is involved in the hydrolysis of starch into simple sugar by acting on its α,1-4 linkages (Hauvermale et al., 2022; Mansoor et al., 2018). Amylase can be acquired from various sources including microorganisms, animals and plants while amylase derived from microorganisms has major advantage as they are stable, less production time and ease in bulk production (Gojiya et al., 2021). Thermostable amylases are of greater concern, as various industrial processes requires high temperature. Similarly thermostable α-amylase is required for poultry feed formulation as the pelleting process of feed requires high temperature treatment where the mesophilic enzyme got denature (Mores et al., 2020; Hussain and Leong, 2023).

Current study evaluates the potential of recombinant α-amylase from *Thermotoga naphthophila* to enhance the starch digestion, to increase the weight gain and to improve the Feed Conversion Ratio (FCR) in poultry broiler chicks.

2. MATERIALS AND METHODS

Chemicals and Reagents

High grade chemicals and reagents used in current study were procured from Merck (Life Sciences, Germany) and Sigma (Life Science, USA).

Ethical Statement

The ethical approval regarding the use of broiler chicks in current research was obtained from ethics committee of University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.

Production of recombinant α-amylase

The pET21a harbouring amylase gene from *T. naphthophila* was utilized for the transformation of BL21CodonPlus (DE3) cells and for the production of recombinant α-amylase under pre optimized conditions. Overnight grown cells were diluted to 1% with fresh LB broth followed by incubation at 37°C till the OD at 660 nm reached to 0.4. The cells were induced with 0.5 mM IPTG followed by 5h post induction incubation under same condition. Cells were harvested by centrifugation at 6000 rpm for 15 min and the obtained cellular pellet was suspended in Tris-HCl buffer (pH 8). Cells were lysed by sonication using Bendelin Sonoplus HD2070 homogenizer. During sonication process, the cellular mixture was kept on ice. The soluble and insoluble fractions after cellular lysis were examined for enzyme activity and its molecular weight by SDS-PAGE (Laemmli, 1970).

Amylase activity assay

Dinitrosalicylic acid (DNS) method was followed for the determination of α-amylase activity (Miller, 1959). Reaction mixture (1 mL) was prepared using 1% starch (125 µL), 50 mM Tris-HCl buffer (pH 8) (105 µL) and purified enzyme (20 µL) followed by incubation at 100°C for 10 min and was promptly shifted on ice to stop the reaction followed by addition of 250 µL DNS reagent and boiling for 5 min. The samples were left to attain room temperature and absorbance was recorded at 540 nm using Double beam UV-Vis spectrophotometer (UV-1601, Shimadzu, USA). One unit of α-amylase activity was defined as the amount of enzyme required to produce one µmol of maltose per min under assay conditions. Standard curve of maltose was used to determine the activity units of amylase.

Biological evaluation of α-amylase activity in poultry birds

I. Enzyme Production

Regarding poultry trials, this α-amylase was produced under optimal conditions using LB medium in batches with a total of 20 L LB medium. The cells after expression were lyzed and soluble part after lysis was utilized as enzyme for the supplementation of poultry feed.

II. Feed formulation

Feed was formulated in an automatic unit at Hitech industries, Shadman, Lahore, Punjab, Pakistan. The composition of feed was same as being used by commercial poultry farms except the supplementation of enzymes (Waris et al., 2024). Five feeds I, II, III, IV and V were prepared. Feed I was for group A, served as negative control and was not supplemented with locally characterized α-amylase. Feed II, III and IV were utilized to feed group B, C and D and were supplemented with 200, 400 and 600 IU/kg of locally characterized α-amylase. Whereas feed V was supplemented with 400 IU/kg with commercially available α-amylase from Rudolf Pakistan (PVT) limited, Doctor's society, Thokar Niaz Baig, Lahore, Punjab, Pakistan, that served as positive control.

III. Poultry Trial

A total of 150, day old broiler chicks of commercial strain were randomly divided into 5 groups, group A was negative control which was fed on feed I, group B, C and D were fed on feed II, III and IV whereas Group E was taken as positive control and was fed on feed V. The trial was conducted for seven weeks, under controlled conditions in control shed located near Muridke, Lahore, Punjab, Pakistan. The birds were having open access to feed and water throughout the tenure and were vaccinated as per standard protocols. Weight of birds and feed intake were recorded as per week basis. Data was also utilized for the estimation of FCR.

Statistical Analysis

Statistical analysis was done using IBM SPSS software. One Way ANOVA, Duncan's multiple range test and T-test were used for data interpretation (Snedecor and Cochran, 1980; Duncan, 1955; Okafor and Anosike, 2012). Post hoc analysis was also performed to examine the pairwise significance (Dunnett, 1955).

3. RESULTS AND DISCUSSION

Aim of the study was to enhance the digestion of starch and non-starch polysaccharide to increase the digestibility and to improve the growth rate of poultry birds by supplementing the feed with locally characterized recombinant α-amylase. The presence of α-amylase in the feed resulted in enhancement of bird growth, increased weight gain and improved FCR. Previous reports are in agreement with the current study (Bassi et al., 2023; Perz et al., 2022; Castro et al., 2020).

The weight gain data demonstrated the increase in body weight of broiler chicks when the feed was supplemented with locally produced recombinant α-amylase. In the first two weeks no significant increase in weight gain was observed as compared to control, however in the third week a significant increase in the weight gain in case of birds fed on feed supplemented with 200, 400 and 600 IU/kg of recombinant α-amylase was observed. That supplementation of feed resulted in increase in weight gain from 402g (Group A) to 429g (Group B), 432g (Group C), 424g (Group D) as compared to 437g (Group E) at the end of third week. Similarly the weight gain data at the end of fourth week depicted an increase in weight gain from 451g (Group A) to 501, 594, 589 and 568g for Group B, C, D and E. The weight gain data at the end of fifth week showed enhanced weight gain from 566g (Group A) to 568, 680, 686 and 596g for Group B, C, D and E. Significant weight gain was recorded at the end of sixth week with an increase in weight from 562g (Group A) to 574, 690, 684 and 643g for Group B, C, D & E and seventh week data demonstrated the weight gain from 596 (Group A) to 589, 690, 694 and 666g for Group B, C, D and E respectively (Table I). The data in Table I clearly depicted the impact of recombinant α-amylase on increased weight gain in broiler chicks and this is in agreement to previous reports (Aderibigbe et al., 2020; Castro et al., 2020; Radhi et al., 2023).

Similarly the FCR value calculated at the end of the 7th week demonstrated the improvement in FCR from 2.17 for Group A (negative control) to 2.13 for Group B (feed supplemented with 200 IU/kg of recombinant α-amylase), 2.01 for Group C (feed supplemented with 400 IU/kg of recombinant α-amylase), 1.95 for Group D (feed supplemented with 600 IU/kg of recombinant α-amylase) (Table II). This showed a clear improvement of FCR from 2.17 (Group A) to 1.95 (Group D). Group D showed significantly enhanced FCR (1.95) as compared to 2.09 for Group E (positive control) supplemented with commercially available α amylase and is used for the supplementation of poultry feed. Previous reports also demonstrated the increase in the FCR due to supplementation for poultry feed with α-amylase (Zhou et al., 2021; Archer, 2024; Sachramm et al., 2021).

Tukey test as Post-hoc analysis confirmed the significance of data (Fig 1) and clearly demonstrated that Group D showed highest impact on the weight gain and FCR values as compared to positive control (Fig 1).

CONCLUSION

The current study concluded that the locally characterized recombinant α-amylase showed strong impact on the growth of poultry birds and resulted in increased weight gain from 596 g (negative control) to 649 g (feed supplemented with 600 IU/kg of α-amylase) and improved FCR from 2.17 to 1.95 respectively. The bulk production of this enzyme at domestic level will be helpful for the fulfilment of local industrial demand for α-amylase and will save huge foreign exchange for the import of this enzyme.

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CONFLICT OF INTEREST DECLARATION

The authors declared no conflict of interest.

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Table I. Effect of α-amylase supplemented diet on weight gain of chicks

Groups	Week 1 (P=0.0053)	Week 2 (P=0.0335)	Week 3 (P=0.0003)	Week 4 (P=0.0003)	Week 5 (P=0.0001)	Week 6 (P=0.0001)	Week 7 (P=0.0304)
A (-ve control)	119.67±6.58	245.00±4.31	402.33±2.03	451.33±2.67	566.00±4.04	562.33±27.17	596.00±4.00
B (200 IU/kg)	137.60±0.31	242.07±0.58	429.00±1.00	501.00±1.53	568.17±0.17	574.50±0.50	589.00±1.00
C (400 IU/kg)	133.43±0.47	232.57±2.59	432.00±1.73	594.77±0.50	680.77±0.82	690.57±3.09	690.27±28.71
D (600 IU/kg)	138.57±0.38	239.47±0.13	424.23±1.38	589.13±1.40	686.40±0.40	684.57±1.94	694.10±25.93
E (+ve control)	124.90±1.84	237.67±1.30	437.43±6.97	568.50±14.85	596.23±15.00	643.40±25.85	666.83±13.17

Table II. The cumulative weight gain feed intake and FCR

Groups	A (negative control)	B (200 IU/kg)	C (400 IU/kg)	D (600 IU/kg)	E (400 IU/kg) Positive control
FCR	2.17	2.13	2.01	1.95	2.09

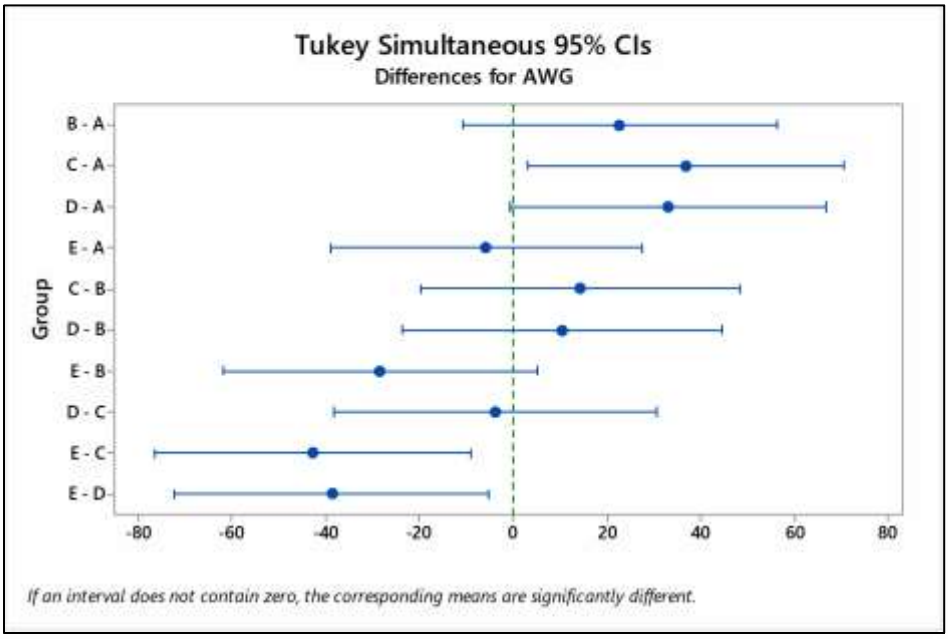

Fig. 1. Post hoc Analysis: Average Weight Gain of group A, B, C, D and E was compared with each other to observe the significance values