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Impact of Locally Characterized Protease from *Geobacillus* SBS 4S on the Growth of Poultry Bird

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WA performed experimental work. M.T. planned and supervised the study and guided for manuscript write-up and editing. MNA, ASH, MDA, SF, SS, BM and MT helped in facilitated the student for the conduction of experiments. MW and ARA helped in manuscript write up. MA helped in data analysis.

Key words
Protease, *Geobacillus* sp. SBS-4S, broiler, Weight gain, Feed supplementation, Evaluation

ABSTRACT

Present study was conducted to determine the efficacy of novel thermostable protease in poultry production. The protease was produced from *Geobacillus* sp. SBS-4S using Luria Bertani medium and it was used as supplement in poultry feed trial. The data were statistically analyzed by Multivariate Analysis of Variance. For feeding, 150 day-old broiler chicks were divided randomly into 5 groups having 30 chicks each. Group A served as negative control, group B, C and D as experimental groups which were supplemented in the basal diet with 2500, 5000 and 7500 IU/kg of locally characterized thermostable protease while group E was supplemented with the commercially available neutral protease (5000 IU/kg) that served as a positive control. The trial lasted for five weeks and during the trial period birds were offered *ad libitum* access to water and feed. The maximal weight gain was recorded when the feed was supplemented with 7500 IU/Kg of locally characterized protease. The evaluation of data of various groups obtained after 3rd, 4th and 5th weeks of trial demonstrated significant change ($P \leq 0.05$) in weight gain, feed up take and feed conversion ratio (FCR) as compared to negative control. Locally produced protease showed promising results as compared to positive control which is being used currently in the poultry industry. The weight gain, feed intake and feed conversion ratio of 1891.54g, 3750g and 1.98 of experimental group D were comparable to 1874.49g, 3740 g and 1.995 in case of positive control. The ability of protease to enhance weight gain, feed consumption and FCR in poultry birds makes it a strong candidate for the replacement of imported counterpart being utilized currently in poultry feed industry.

INTRODUCTION

Poultry sector is one of the fast growing industries of Pakistan that is playing a significant role in bridging gap in meat demands at economical prices (Hussain *et al*., 2015). However, the increased demand of feed ingredients has resulted in high feed cost and narrowing down the profit margin (Thirumalaisamy *et al*., 2016). The plant source proteins derived from grains and soyabean contain various complex molecules which are not being hydrolyzed by the mono-gastric animals due to low or unavailability of enzymes. This problem can be overcomed by supplementing the poultry feed with hydrolytic enzymes, responsible for the breakdown of complex to simple absorbable monomeric components.

The research on utilization of exogenous enzymes in poultry feed to decrease the total nutrient excretion and to enhance nutrient utilization by improving the digestive

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process has been ongoing for the past several years (Leeson and Summers, 2005; Cambell and Bedford, 1992; Rada *et al*., 2016; Ganapathi *et al*., 2014; Seskeviciene *et al*., 1999; Toghyani *et al*., 2017). During the first week, the broiler chicken's gastro intestinal tract is not fully developed. Therefore, the optimal level of enzymatic secretions is not gained (Mahagna *et al*., 1995). This deficiency of endogenous enzymes is further augmented due to high demand of amino acids and fast passage rate by the body of broiler chicks (Doskovic *et al*., 2013).

There are reports on digestibility of amino acids and crude proteins indicating that considerable amounts of proteins (18-20%) travel through the gastrointestinal tract without being digested completely (Lemme *et al*., 2004; Wang and Parsons, 1998; Angel *et al*., 2011; Applegate and Angel, 2014). This aggravated amino acid demand can be fulfilled either by utilizing higher protein content or by supplementing the feed with protease for aiding the endogenous proteolytic system for the enhanced digestion of dietary protein. Therefore, poultry feed has been supplemented with exogenous proteases to improve nutritive value of poultry diet (Cowieson and Roos, 2016). The use of synthetic enzymes significantly decrease the amounts of excreted nutrients such as phosphorous and nitrogen and stimulate the digestibility of nutrients resulting in reduction of anti-nutritional impact of complexation of phosphorous and amino acids (Sabir *et al*., 2018; Khalid *et al*., 2019; Costa *et al*., 2006; Ghazi *et al*., 2002).

The feed supplemented with proteases significantly improves the body weight, feed conversion ratio and protein digestibility of feed stuffs in poultry chicks (Oxenboll *et al*., 2011; Fru-Nji *et al*., 2011; Puente and Lopez, 2004; Angel *et al*., 2011; Freitas *et al*., 2011; Bolan *et al*., 2010; Ndazigaruye *et al*., 2019; Mahmood *et al*., 2017). The increased digestibility of poultry birds increases the possibility of reduced nitrogen excretion by the bird's body, reduced ammonia production from the excreta of animals, decrease the content of protein wasted and cost of production (Oxenboll *et al*., 2011). The addition of protease to the poultry feed shows more prominent and positive effects on feed conversion ratio (FCR), which leads to the significant environmental benefits (Angel *et al*., 2011; Fru-Nji *et al*., 2011). Many scientists have worked on the characterization of proteases for the poultry feed industry but unfortunately none of the protease is available in the market for the fulfilment of local industrial demand (Ali *et al*., 2016; Aftab *et al*., 2006; Mukhtar and Haq, 2012; Ahmad *et al*., 2020). Moreover, Pakistan is spending a huge foreign exchange for the import of the enzymes per annum. The present study was planned to utilize the locally characterized protease from *Geobacillus* sp. SBS-4S for the evaluation of its efficacy on poultry bird's growth. This protease has been characterized which showed its optimal activity at 65°C and pH 9 in the presence of Mn^{2+} (Ahmad *et al*., 2020).

MATERIALS AND METHODS

Protease production

The protease was produced under pre-optimized conditions from 34 liters of LB medium supplemented with 2% yeast extract and 5% wheat bran, inoculated with the overnight grown *Geobacillus* sp. SBS-4S cells followed by incubation at 60°C for 18h under shaking conditions at 120rpm in 5 batches. The microbial growth was centrifuged and supernatant was assessed for protease activity (Pant *et al*., 2015; Sharma *et al*., 2017; Catara *et al*., 2003). Protein contents were calculated by Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Feed formulation

Broiler feed was formulated as per recommendations of American National Research Council (NRC, 2014) and is being used by the poultry industry (corn, 58.0%; soya bean meal 46, 29.5%; rice polish 14, 7.50%; calcium carbonate ($CaCO_3$), 0.95%; oil, 2.00%; bone ash, 0.75%; DCP (dicalcium phosphate), 0.50%; vitamin premix, 0.30%; L-HCl (lysine HCl), 0.25%; sodium bicarbonate ($NaHCO_3$), 0.17%; DLM (DL-methionine), 0.15%). The poultry feed was prepared in an automated unit at Crescent Feeds and Allied Products, Sundar Sharif, Lahore, Punjab, Pakistan. Five diets were prepared. Diet I was not supplemented with the protease (negative control) while diets II, III and IV were supplemented with protease 2500, 5000 and 7500 IU/kg of feed, respectively. Diet V was supplemented with 5000 IU/kg of commercially available protease as positive control.

Feeding trials on broiler chicks

Feeding trial was conducted under controlled environment at Dua Poultry Farms, Niaz Kot, Kala Shah Kaku, Tehsil Ferozewala, District Sheikhupura, Punjab, Pakistan in collaboration with Crescent Feeds and Allied Products, Sundar Sharif, Lahore, Punjab, Pakistan. For feeding trial, 150 one-day-old broiler chicks with an average weight of 39g per chick were purchased from a commercial hatchery and randomly divided into 5 groups A, B, C, D and E. Each group consisted of 30 birds, having 3 replicates of 10 birds each. Group A was declared as negative control and was fed on diet without protease. Group B, C and D were fed on diets II, III and IV respectively, which were supplemented with 2500, 5000 and 7500 IU/kg of locally produced protease. Group E acted as positive control and was fed on diet V supplemented with commercially available protease (5000

IU/kg of feed). Water and feed were given *ad libitum* during the feeding trial for a period of 5 weeks. The body weight, feed consumption and feed conversion ratio were recorded on week basis (Sabir *et al*., 2018; Rahman *et al*., 2014).

Statistical analysis

The obtained data was analyzed statistically by Multivariate Analysis of Variance using SPSS software (Okafor and Anosike, 2012). The results obtained were represented as significance of differences between means calculated by Fisher's Least Significant Difference test. The differences were significant at $P \leq 0.05$ (Steel *et al*., 1997).

RESULTS

Feeding trials on poultry birds demonstrated that supplementation of broiler feed with locally characterized protease significantly enhanced the weight gain and feed consumption of poultry birds with improved feed conversion ratio. The weight gain data regarding 1st and 2nd week of trials showed significant increase in weight gain of various groups fed on diet supplemented with locally characterized protease as compared to negative control (Group A). Significant impact on weight gain of birds was recorded by the end of 3rd, 4th and 5th weeks of experiment (Table I). Comparison of group A (negative control) with group C in the 3rd, 4th and 5th weeks of trials demonstrated the weight gain from 831.66±0.76 to 857.41±0.56g, 1256.35±0.85 to 1291.45±0.74g and 1744.52±0.94 to 1847.55±0.95g, respectively when the poultry diet was supplemented with protease 5000 IU/kg of feed. The comparison of weight gain data of group D with that of group A, B and C demonstrated a boosting effect of locally produced protease on weight gain in poultry birds (Table I).

The increase in protease concentration from 5000 IU/kg (group C) to 7500 IU/kg (group D) showed significant enhancement regarding weight gain and feed consumption. Supplementation of feed with protease enhanced the digestive ability and feed consumption of birds. Comparative analysis indicated the improved feed consumption with the increase in protease concentration. The results demonstrated that group B, C and D utilized 3710, 3730 and 3750 g of feed as compared to group A which utilized 3680 g of feed by the end of 5 weeks of trials. Same pattern of improvement of FCR was recorded in the groups fed on feed supplemented with locally produced protease. The FCR value was improved from 2.11 (group A) to 1.98 (group D) (Table II). A comparison of group D and E revealed the better performance of locally produced protease as compared to enzyme currently being used in the poultry feed industry. Multivariate analysis of variance demonstrated the significant results for various tests (Table III) which supports the positive impact on weight gain due to supplementation of feed with local protease. Multiple comparative analysis among the weeks with respect to various groups showed significant results (Tables III, Supplementary Table I).

Table I. Effect of protease supplementation on weight of poultry chicks during feeding trials.

Groups	1st Week (0.000***)	2nd Week (0.000***)	3rd Week (0.000***)	4th Week (0.000***)	5th Week (0.000***)
A (-ve control)	164.68 ± 0.96^{e}	448.49 ± 0.66^{e}	831.66 ± 0.76^{e}	1256.35 ± 0.85^{e}	1744.52 ± 0.94^{e}
B (2500 IU/kg)	171.56 ± 0.68^{d}	458.49 ± 0.68^{d}	847.42 ± 0.80^{d}	1269.44 ± 0.90^{d}	1826.60 ± 0.66^{d}
C (5000 IU/kg)	175.57 ± 0.73^{c}	467.43 ± 0.74^{c}	857.41 ± 0.56^{c}	1291.45 ± 0.74^{c}	1847.55 ± 0.95^{c}
D (7500 IU/kg)	184.43 ± 0.73^{a}	485.49 ± 0.81^{a}	887.52 ± 0.79^{a}	1356.50 ± 0.68^{a}	1891.54 ± 0.89^{a}
E (5000 IU/kg) (+ive control)	183.31 ± 0.64^{b}	473.59 ± 0.70^{b}	872.42 ± 0.78^{b}	1341.19 ± 0.93^{b}	1874.49 ± 0.75^{b}

*P values indicate the significance among groups of each week. **Means that do not share a letter i.e., a, b, c, d and e are significantly different. Group A, negative control; Group B, 2500 IU/kg; Group C, 5000 IU/kg; Group D, 7500 IU/kg; Group E, +ve control.

Table II. Effect of protease on average feed intake, feed conversion ratio and body weight during feeding trials.

Groups	A	B	C	D	E
Average feed intake (g)	3680	3710	3730	3750	3740
Overall body weight (g)	$1744.52^{e}\pm0.94$	$1826.60^{d}\pm0.66$	$1847.55^{c}\pm0.95$	$1891.54^{a}\pm0.89$	$1874.49^{b}\pm0.75$
Feed conversion ratio (FCR)	2.11^{e}	2.031^{d}	2.019^{c}	1.98^{b}	1.995^{a}

Group A, negative control; Group B, 2500 IU/kg; Group C, 5000 IU/kg; Group D, 7500 IU/kg; Group E, +ve control.

Table III. Multivariate analysis of variance for multivariate tests.

Effect		Value	F	Hypothesis df	Errordf	Sig.
Intercept	Pillai's trace	1.000	20606492.318[b]	5.000	141.000	.000
	Wilks' lambda	.000	20606492.318[b]	5.000	141.000	.000
	Hotelling's trace	730726.678	20606492.318[b]	5.000	141.000	.000
	Roy's largest root	730726.678	20606492.318[b]	5.000	141.000	.000
	Pillai's trace	1.647	20.151	20.000	576.000	.000
	Wilks' lambda	.003	119.073	20.000	468.594	.000
	Hotelling's trace	178.024	1241.714	20.000	558.000	.000
	Roy's largest root	177.003	5097.697[c]	5.000	144.000	.000

a, Design: Intercept + Groups; b, Exact statistic; c, The statistic is an upper bound on F that yields a lower bound on the significance level.

DISCUSSION

Proteases play essential role in the maturation, working and degradation of proteins in the cell and are key component of cell (Douglas *et al*., 2000; Ghazi *et al*., 2002). Proteases are also responsible for the removal of structural proteins in the cell wall polysaccharides which allow faster access to other catabolic enzymes which results in better digestion (Colombatto and Benuchemin, 2009).

Poultry trial confirmed the ability of locally produced protease to improve the digestibility of feed, as supplementation of feed boosted the weight gain and feed uptake of the broiler birds. The comparative analysis of data regarding weight gain of chicks from groups B, C and D with respect to group A (Table I) clearly demonstrated an increase in weight gain with a maximum weight gain of 1891.54±0.89 g (group D) which was comparable to 1874.49±0.75 g (group E) fed on feed supplemented with commercially available protease. These results are similar to previous findings (Vieira *et al*., 2013), who reported the role of protease for the enhancement of birds weight gain. Similar results were demonstrated when poultry birds were fed with diet supplemented with protease from *Bacillus subtilis* C-3102 (Hooge *et al*., 2004).

Weight gain data indicated that protein digestibility, weight gain and feed utilization of poultry birds were improved with the increase in concentration of enzyme. The weight gain and FCR values of 1891.54±0.89g and 1.98 were recorded, respectively for 7500 IU/kg of locally produced protease as compared with results of 1874.49±0.75g and 1.995 for 5000 IU/kg of commercially available protease being imported to the country with significant p value ≤0.05. These results are in agreement with previous reports on the improvement of FCR due to supplementation of feed with protease (Angel *et al*., 2011; Ajayi and Imouokhome, 2015; Aureli *et al*., 2010; Castanon and Marquardt, 1989; Fru-Nji *et al*., 2011; Kocher *et al*., 2015; Ndazigaruye *et al*., 2019; Park and Kim, 2018; Rada *et al*., 2013; Sonu *et al*., 2018).

CONCLUSION

The locally characterized protease from *Geobacillus* sp. SBS-4S has strong potential for enhanced protein digestion and for the improved weight gain, feed consumption and FCR value in broiler chicks. The domestic production of this protease may result in low cost availability of the enzyme that can replace the imported counterpart being utilized currently in poultry feed industry.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20220324050332

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Impact of Locally Characterized Protease from *Geobacillus* SBS 4S on the Growth of Poultry Bird

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Supplemntary Table I. Multiple comparisons analysis by least significant difference.

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Week_1	A	B	-6.8790*	.52465	.000	-7.9160	-5.8420
		C	-10.8923*	.52465	.000	-11.9293	-9.8554
		D	-19.7530*	.52465	.000	-20.7900	-18.7160
		E	-18.6327*	.52465	.000	-19.6696	-17.5957
	B	A	6.8790*	.52465	.000	5.8420	7.9160
		C	-4.0133*	.52465	.000	-5.0503	-2.9764
		D	-12.8740*	.52465	.000	-13.9110	-11.8370
		E	-11.7537*	.52465	.000	-12.7906	-10.7167
	C	A	10.8923*	.52465	.000	9.8554	11.9293
		B	4.0133*	.52465	.000	2.9764	5.0503
		D	-8.8607*	.52465	.000	-9.8976	-7.8237
		E	-7.7403*	.52465	.000	-8.7773	-6.7034

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Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
	D	A	19.7530*	.52465	.000	18.7160	20.7900
		B	12.8740*	.52465	.000	11.8370	13.9110
		C	8.8607*	.52465	.000	7.8237	9.8976
		E	1.1203*	.52465	.034	.0834	2.1573
	E	A	18.6327*	.52465	.000	17.5957	19.6696
		B	11.7537*	.52465	.000	10.7167	12.7906
		C	7.7403*	.52465	.000	6.7034	8.7773
		D	-1.1203*	.52465	.034	-2.1573	-.0834
Week_2	A	B	-10.0027	992.16420	.992	-1970.9751	1950.9698
		C	-18.9373	992.16420	.985	-1979.9098	1942.0351
		D	-36.9990	992.16420	.970	-1997.9714	1923.9734
		E	-1593.7843	992.16420	.110	-3554.7568	367.1881
	B	A	10.0027	992.16420	.992	-1950.9698	1970.9751
		C	-8.9347	992.16420	.993	-1969.9071	1952.0378
		D	-26.9963	992.16420	.978	-1987.9688	1933.9761
		E	-1583.7817	992.16420	.113	-3544.7541	377.1908
	C	A	18.9373	992.16420	.985	-1942.0351	1979.9098
		B	8.9347	992.16420	.993	-1952.0378	1969.9071
		D	-18.0617	992.16420	.986	-1979.0341	1942.9108
		E	-1574.8470	992.16420	.115	-3535.8194	386.1254
	D	A	36.9990	992.16420	.970	-1923.9734	1997.9714
		B	26.9963	992.16420	.978	-1933.9761	1987.9688
		C	18.0617	992.16420	.986	-1942.9108	1979.0341
		E	-1556.7853	992.16420	.119	-3517.7578	404.1871
	E	A	1593.7843	992.16420	.110	-367.1881	3554.7568
		B	1583.7817	992.16420	.113	-377.1908	3544.7541
		C	1574.8470	992.16420	.115	-386.1254	3535.8194
		D	1556.7853	992.16420	.119	-404.1871	3517.7578
Week_3	A	B	-15.7647	2582.72859	.995	-5120.4233	5088.8940
		C	-25.7500	2582.72859	.992	-5130.4086	5078.9086
		D	-5933.0237*	2582.72859	.023	-11037.6823	-828.3650
		E	-40.7603	2582.72859	.987	-5145.4190	5063.8983
	B	A	15.7647	2582.72859	.995	-5088.8940	5120.4233
		C	-9.9853	2582.72859	.997	-5114.6440	5094.6733
		D	-5917.2590*	2582.72859	.023	-11021.9176	-812.6004
		E	-24.9957	2582.72859	.992	-5129.6543	5079.6630
	C	A	25.7500	2582.72859	.992	-5078.9086	5130.4086
		B	9.9853	2582.72859	.997	-5094.6733	5114.6440
		D	-5907.2737*	2582.72859	.024	-11011.9323	-802.6150
		E	-15.0103	2582.72859	.995	-5119.6690	5089.6483
	D	A	5933.0237*	2582.72859	.023	828.3650	11037.6823

Table continued in next page............

Dependent variable	(I) Groups	(J) Groups	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
		C	5907.2737*	2582.72859	.024	802.6150	11011.9323
		E	5892.2633*	2582.72859	.024	787.6047	10996.9220
	E	A	40.7603	2582.72859	.987	-5063.8983	5145.4190
		B	24.9957	2582.72859	.992	-5079.6630	5129.6543
		C	15.0103	2582.72859	.995	-5089.6483	5119.6690
		D	-5892.2633*	2582.72859	.024	-10996.9220	-787.6047
Week_4	A	B	-13.0980*	2.24144	.000	-17.5281	-8.6679
		C	-35.1073*	2.24144	.000	-39.5375	-30.6772
		D	-100.1523*	2.24144	.000	-104.5825	-95.7222
		E	-84.8450*	2.24144	.000	-89.2751	-80.4149
	B	A	13.0980*	2.24144	.000	8.6679	17.5281
		C	-22.0093*	2.24144	.000	-26.4395	-17.5792
		D	-87.0543*	2.24144	.000	-91.4845	-82.6242
		E	-71.7470*	2.24144	.000	-76.1771	-67.3169
	C	A	35.1073*	2.24144	.000	30.6772	39.5375
		B	22.0093*	2.24144	.000	17.5792	26.4395
		D	-65.0450*	2.24144	.000	-69.4751	-60.6149
		E	-49.7377*	2.24144	.000	-54.1678	-45.3075
	D	A	100.1523*	2.24144	.000	95.7222	104.5825
		B	87.0543*	2.24144	.000	82.6242	91.4845
		C	65.0450*	2.24144	.000	60.6149	69.4751
		E	15.3073*	2.24144	.000	10.8772	19.7375
	E	A	84.8450*	2.24144	.000	80.4149	89.2751
		B	71.7470*	2.24144	.000	67.3169	76.1771
		C	49.7377*	2.24144	.000	45.3075	54.1678
		D	-15.3073*	2.24144	.000	-19.7375	-10.8772
Week_5	A	B	-12.0850*	.58422	.000	-13.2397	-10.9303
		C	-33.0293*	.58422	.000	-34.1840	-31.8746
		D	-77.0273*	.58422	.000	-78.1820	-75.8726
		E	-59.9757*	.58422	.000	-61.1304	-58.8210
	B	A	12.0850*	.58422	.000	10.9303	13.2397
		C	-20.9443*	.58422	.000	-22.0990	-19.7896
		D	-64.9423*	.58422	.000	-66.0970	-63.7876
		E	-47.8907*	.58422	.000	-49.0454	-46.7360
	C	A	33.0293*	.58422	.000	31.8746	34.1840
		B	20.9443*	.58422	.000	19.7896	22.0990
		D	-43.9980*	.58422	.000	-45.1527	-42.8433
		E	-26.9463*	.58422	.000	-28.1010	-25.7916
	D	A	77.0273*	.58422	.000	75.8726	78.1820
		B	64.9423*	.58422	.000	63.7876	66.0970
		C	43.9980*	.58422	.000	42.8433	45.1527
		E	17.0517*	.58422	.000	15.8970	18.2064
	E	A	59.9757*	.58422	.000	58.8210	61.1304
		B	47.8907*	.58422	.000	46.7360	49.0454
		C	26.9463*	.58422	.000	25.7916	28.1010
		D	-17.0517*	.58422	.000	-18.2064	-15.8970

Based on observed means. The error term is Mean Square (Error) = 5.120. *, The mean difference is significant at the .05 level.