



Effect of Beta-Xylanase and Cysteine Protease Enzymes in Diets on Performance of Kadaknath Birds

Sandeep Gupta* and M.K. Mehta

Department of Animal Nutrition, College of Veterinary Science & A.H., NDVASU, Mhow, INDIA

**Corresponding author: S Gupta: drsandeepvet@gmail.com*

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ABSTRACT

India is home to nineteen breeds of indigenous fowl including one well-known native breed “Kadaknath” or “Kalamasi” meaning a fowl having black flesh. Although the Kadaknath breed has many unique characteristics, it has been neglected because of its poor production potential. In recent trends, the higher use of single activity enzymes like xylanase and protease in commercial poultry feed for improving their performance can also improve performance in Kadaknath breed. Efforts for attaining higher body weight in short duration is still a subject of research for this breed. An experiment of three months was conducted with total Two hundred Kadaknath chicks which were randomly divided into four equal groups with five replicates and each replicate consisted of 10 chicks. Experimental control diet (T_0) for pre starter, starter and finisher birds consisted of maize, soybean meal and feed additives based on BIS (2007) for broilers. Treatment diet one (T_1) was supplemented with 0.1 kg/ton beta-xylanase enzyme (min. 16000 bxu/kg) with reducing dietary ME 100 Kcal/kg and 1.75% CP, treatment diet two (T_2) was supplemented with 0.25 kg/ton cysteine protease enzyme (min. 32500 pu/kg) with reducing dietary ME 50 Kcal/kg and 4% CP and Treatment diet three (T_3) was supplemented with both xylanase and protease on same dose in T_2 and T_3 respectively with reducing dietary ME 150 Kcal/kg and 5.75% CP. The average body weight gain and average feed intake were recorded fortnightly. A metabolic trial was conducted during 6th fortnight of growth period. The average body weight, feed consumption and FCR were not significantly ($P>0.05$) different among all treatments. Crude protein digestibility was observed significantly ($P>0.05$) higher in protease supplemented group where as dry matter and crude fibre digestibility were observed significantly ($P>0.05$) higher in T_1 . In the above study, it was concluded that the growth performance was similar in xylanase, protease and combination of both supplemented group with reducing energy and protein in comparison to control group. Combination of xylanase and protease was more profitable in rearing of Kadaknath birds.

Keywords: Kadaknath, Xylanase enzyme, Cysteine protease, Growth, Profitability

Indigenous breed “Kadaknath” is well known for delicious black flesh with black beak, feathers, comb and shank in India. It is native of Jhabua district of Madhya Pradesh. In all the varieties of Kadaknath most of the internal organs exhibit intense black coloration, which is due to the deposition of melanin pigment in the connective tissue of the organs and in the dermis (Rao and Thomas, 1984). The bird is very popular due to its special capabilities such as adaptability to local environment, resistance to certain diseases, meat quality and many other criteria specific to breed type (Rao and Thomas, 1984). Mohan *et al.* (2008) reported that the meat of the Kadaknath breed contains a high percentage (25.47 %) of protein and is believed to have

aphrodisiac properties. Although the Kadaknath breed has many unique characteristics, it has been neglected because of its poor production potential. Kadaknath is reared by most of the tribes in backyard system of farming without any vaccination and hygiene. It is offered only kitchen waste and small amount of grains, therefore it's growth rate is very low and mortality rate is high. Mondal *et al.* (2007) reported that Kadaknath × Brown Cornish cross attained maximum body weight (1023 g) in 10 weeks. Efforts for attaining higher body weight in short duration is still a subject of research for this breed.

At present the nutrient requirements of Kadaknath is not well established. Central Avian Research Institute (CARI)



and other National poultry organizations recommend same Bureau of Indian Standard (BIS) diet for all indigenous breeds which do not correlate with the performance of individual breed. In recent trends, the higher use of enzymes in commercial poultry feed for improving their performance can also improve performance in Kadaknath breed (Panda *et al.*, 2012).

Proven benefits of feed enzymes include improved feed efficiency, reduced feed cost, improved digestion and absorption of nutrients, improved uniformity within flocks and better maintenance of gut health (Barletta, 2010). Supplementing the feed with specific enzyme improves the nutritional value of feed ingredients, increasing the efficiency of digestion. Feed enzymes help in breakdown of anti-nutritional factors (e.g. fibre, phytate) that are present in many feed ingredients. Use of exogenous xylanase has two well documented benefits, firstly releasing encapsulated nutrients such as starch and protein from the cells and secondly reducing the viscosity of the digesta, both leading to improvement in digestibility (Choct, 2006 and Mirzaie *et al.*, 2012). The breakdown of non-starch polysaccharides by beta-xylanase can also have a beneficial effect on the gut microflora by creating conditions that encourage beneficial bacteria through reduction in viscosity and production of small oligomers that can be used by the beneficial bacteria in the lower gut (Bedford, 2000).

Soybean meal is highly acceptable vegetable protein source in poultry industry, due to this demand of soybean meal is always high. But the increased use of soybean in human being is major challenge of availability for poultry industry (FAOSTAT, 2010). Exogenous proteases are used to increase the hydrolysis of proteins in the feed, including hydrolysis of proteinaceous anti-nutrients such as trypsin inhibitors (Caine *et al.*, 1998), resulting in improved digestibility of protein and amino acids (Cowieson and Adeola, 2005). Most of vegetable protein source have 80-90 percent of digestible protein. Whereas the animal protein sources like meat-bone meal, fish meal are having less than 70 percent digestible protein. Digestibility of protein source depends upon the action of secretory endogenous protease enzymes like pepsin, trypsin, chymotrypsin etc. High protein diet needs exogenous source of proteases which can help in breakdown of feed proteins that can be more digested by the animal. Cysteine protease is peptidase of C1 family. It contains 212 amino acid residue chains. It

consists of a single polypeptide chain with three disulfide bridges and a sulfhydryl group necessary for activity of the enzyme (Grzonka *et al.*, 2001). Kadaknath is a bird of choice and is having a better market value as compared to other birds. Therefore, their production in short duration is biggest challenge for Indian poultry nutritionist. Since this is a slow growing breed, this type of work becomes a need for today. Higher acceptability of specific enzymes such as xylanase and protease in commercial poultry farming is because of their positive effect on growth performance resulting in to a short duration for optimum growth of broilers. The similar effect can be expected in indigenous bird like Kadaknath because this breed is adopted to different climatic condition of India.

MATERIALS AND METHODS

Total two hundred straight run, day old Kadaknath chicks belonging to same hatch were weighed individually and randomly distributed into four treatment groups. Each group consisting of five replicates of ten chicks each. The chicks were housed in deep litter system. Corn-soybean meal based feed was formulated for broiler using BIS (2007) and analyzed for proximate nutrients (AOAC, 2012) which are presented in Table 1 and 2. The duration of the experiment was three months. The standard pre-starter mash (0-15 days), starter mash (16-45 days) and finisher mash (45 days to 90 days) feed were provided *ad libitum* to all the birds using BIS (2007) along with clean drinking water. A weighed quantity of feed was offered to each group of birds two times a day. The feed left over were collected and weighed at fortnightly interval to arrive at fortnightly feed consumption of each group. Experimental control diet (T_0) for pre starter, starter and finisher birds consisted of maize, soybean meal and feed additives based on BIS (2007) for broilers. Treatment diet one (T_1) was supplemented with 0.1 kg/ton beta-xylanase enzyme (min. 16000 bxu/kg) with reducing dietary ME 100 Kcal/kg and 1.75% CP; treatment diet two (T_2) was supplemented with 0.25 kg/ton cysteine protease enzyme (min. 32500 pu/kg) with reducing dietary ME 50 Kcal/kg and 4% CP and Treatment diet three (T_3) was supplemented with both xylanase and protease on same dose in T_2 and T_3 , respectively with reducing dietary ME 150 Kcal/kg and 5.75% CP. The average body weight gain, average feed intake and feed conversion ratio were recorded fortnightly.

Table 1: Composition of feed ingredients and additives/supplements (kg/ton) in the rations

<i>Ingredients</i>	T₀			T₁			T₂			T₃		
	<i>Prestarter</i>	<i>Starter</i>	<i>Finisher</i>	<i>Prestarter</i>	<i>Starter</i>	<i>Finisher</i>	<i>Prestarter</i>	<i>Starter</i>	<i>Finisher</i>	<i>Prestarter</i>	<i>Starter</i>	<i>Finisher</i>
Maize	568	575	613	596	612	650	608	615	653	604	648	690
Soybean meal	340	318	268	327	303	253	314	291	242	308	277	227
Calcite	6	6	6	11	6	6	6	6	6	21	8	6
Di calcium phosphate	4	4	5	4	4	5	4	4	5	4	4	5
Meat & Bone meal	50	50	50	50	50	50	50	50	50	50	50	50
Oil	19	35	46	0	13	24	5	21	32	0	0	10
DL-Methionine	1.8	2.1	2.1	1.8	2.1	2.1	1.4	1.7	1.7	1.5	1.8	1.8
L-Lysine	0.9	0.4	0.0	1.1	0.6	0.0	1.2	0.7	0.0	1.3	1.0	0.1
Soda Bicarbonate	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Trace Minerals	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Vitamin Premix	0.60	0.50	0.50	0.60	0.50	0.50	0.60	0.50	0.50	0.60	0.50	0.50
Acidifiers	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Herbal choline	1.00	1.00	1.20	1.00	1.00	1.20	1.00	1.00	1.20	1.00	1.00	1.20
Liver tonic	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Betaine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Emulsifier	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Toxin Binder	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Antidiarrheal	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Xylanase	0.00	0.00	0.00	0.10	0.10	0.10	0.00	0.00	0.00	0.10	0.10	0.10
Protease	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.25	0.25	0.25	0.25	0.25
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Table 2: Chemical composition of rations

Nutrients	T₀			T₁			T₂			T₃		
	<i>Pre-Starts</i>	<i>Starter</i>	<i>Finisher</i>	<i>Pre-starter</i>	<i>Starter</i>	<i>Finisher</i>	<i>Pre-starter</i>	<i>Starter</i>	<i>Finisher</i>	<i>Pre-starter</i>	<i>Starter</i>	<i>Finisher</i>
Crude Protein (%)	22.96	21.94	20.06	22.61	21.56	19.43	22.17	21.09	19.07	21.76	20.72	18.68
ME (Mcal/Kg) *	3.00	3.10	3.20	2.90	3.00	3.10	2.95	3.05	3.15	2.88	2.95	3.05
Calorie protein ratio*	130.43	140.91	160.00	128.04	138.57	157.76	133.34	144.39	164.72	131.95	142.15	162.46
Crude Fibre (%)	3.75	3.63	3.45	3.71	3.69	3.42	3.71	3.59	3.41	3.66	3.61	3.43
Ether extract (%)	4.43	5.98	7.14	2.61	3.87	5.05	3.12	4.67	5.84	2.62	2.72	3.74
Amino acids												
Lysine* (%)	1.30	1.20	1.03	1.29	1.19	1.00	1.26	1.16	0.97	1.25	1.15	0.95
Methionine* (%)	0.53	0.55	0.52	0.54	0.55	0.53	0.49	0.50	0.48	0.49	0.51	0.48
Minerals												
Calcium* (%)	1.00	1.00	1.00	1.14	1.00	1.00	1.00	1.00	1.00	1.50	1.05	1.00
Avail. phosphorus* (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

* Calculated values



A metabolic trial was conducted during 6th fortnight of growth period for analysed dry matter, crude protein and crude fibre digestibility. The data generated through the experimental period were subjected to statistical analysis by General Linear Models (GLM) procedure of the Completely Randomized Design using software the Statistical Analysis System (SAS) to study the effect of treatment on various parameters (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The Kadaknath birds were fed on basal diets as control group (T_0) using BIS (2007) standard for broiler feed. The beta-xylanase (T_1), cysteine protease (T_2) alone and beta-xylanase and cysteine protease both (T_3), supplemented groups with basal diet were used with reducing level of energy and protein according to their dose. The data regarding overall growth are presented in Table 3.

Table 3: Overall growth of experimental birds

Treatment	Body weight (g)	Feed consumption (g)	FCR
T_0	901.67±66.32	2785.10±348.75	3.18±0.32
T_1	867.00±53.83	2627.80±129.23	3.12±0.35
T_2	932.22±65.94	2645.20±157.67	2.92±0.23
T_3	929.63±73.66	2678.19±250.01	2.96±0.21

The growth parameters include body weight, feed consumption and feed conversion ratio were not significantly different ($P > 0.05$) between treatments. However, numerically improved body weight and FCR were recorded in cysteine protease enzyme supplemented group. It may be due to supplementation of single activity enzymes which compensate reduced level of energy and protein in the diets of T_1 , T_2 and T_3 in comparison to T_0 . The above results are in agreement with the findings of Zanella *et al.* (1999), Cafe *et al.* (2002), Mathlouthi *et al.* (2002), Kocher *et al.* (2003), Odetallah *et al.* (2005), Fru-Nji *et al.* (2011), Abou El-wafa *et al.* (2013) and Stefanello *et al.* (2015) who conducted the experiments on different age groups of broiler chicks with single activity xylanase, protease and combination of both with reducing level of energy and protein in comparison to basal diet and found similar non-significantly ($P > 0.05$) different results between the groups. It might be due to

the fact that reduced energy and crude protein have been compensated by xylanase and protease supplementation for improving nutrient digestibility. Abou El-wafa *et al.* (2013) concluded that xylanase enzyme (16000 u/kg) added to corn-soybean meal based diet could reduce the dietary energy level about 100 kcal/kg, as no significant ($P > 0.05$) difference in growth performance was observed. Stefanello *et al.* (2015) also reported non-significantly ($P < 0.05$) different feed consumption in xylanase and protease supplemented groups in comparison to control. Kocher *et al.* (2003) reported non-significant FCR in an experiment with xylanase and protease supplemented diet in low energy group in comparison to control. The results might be attributed to non-significantly ($P < 0.05$) different body weight and feed consumption in xylanase and protease supplemented groups. Similarly, Odetallah *et al.* (2005) observed that supplementing the low protein (LP) diet with enzyme did not improve the performance of the chicks to a level equivalent to that of the control group. However, supplementing the LP diet with the 0.10 % enzyme (wt/wt) level significantly ($P < 0.05$) improved performance of chicks over that the LP diet. Wang *et al.* (2006) reported that versazyme supplementation numerically improved FCR in low protein diet.

The result obtained during metabolic trial for nutrient utilization are presented in Table 4. Crude protein digestibility was significantly ($P < 0.05$) higher recorded in protease supplemented group. Similar results of improved crude protein digestibility in protease supplemented diet were reported by Zanella *et al.* (1999), Fru-Nji *et al.* (2011) and Romero *et al.* (2013) in commercial broilers.

Table 4: Average nutrient utilization during metabolic trial of experimental birds

Treatment	Dry matter (%)	Crude protein (%)	Crude fibre (%)
T_0	59.91±4.18 ^{AB}	48.04±10.12 ^{AB}	30.89±10.10 ^{AB}
T_1	64.80±3.55 ^A	49.49±7.69 ^{AB}	40.76±7.85 ^A
T_2	58.85±5.75 ^{AB}	56.79±6.71 ^A	22.28±8.89 ^B
T_3	56.02±3.43 ^B	47.11±10.16 ^B	26.81±10.35 ^{AB}

Total tract apparent retention (TTAR) of N increased significantly ($P < 0.05$) with PXA supplementation versus the control group, whereas XA supplementation did not cause a significant change from control diet in

an experiment conducted by Romero *et al.* in year 2013. Fru-Nji *et al.* (2011) also reported that the Ronozyme Pro-act (RPA) significantly ($P < 0.05$) improved protein digestibility by about 8% and total fat digestibility by 3%. RPA is pure protease and does not have any lipase activity, the improvement of fat digestibility recorded is most probably a secondary effect of protein degradation. By degrading large protein molecules in a chyme complex, there might be better access to the total surface area of the lipid molecules for micelle formation. The overall CP digestibility (ileal) brought about by enzyme supplementation was increased 2.9% (absolute value, 3.6% relative increase) in trial of Zanella *et al.* (1999).

Table 5: Economics of experimental birds

Treatments			T ₀	T ₁	T ₂	T ₃
Chick cost	(₹)		30	30	30	30
Feed intake and cost of feed/bird	0-2 weeks	Feed intake (g)	136	137	138	138.4
		Feed cost (₹)	22.45	21.37	21.33	20.9
	3-6 weeks	Feed intake (g)	956.37	938.45	928.69	920.3
		Feed cost (₹)	22.77	21.56	21.65	20.51
	7-12 weeks	Feed intake (g)	1692.66	1552.37	1578.48	1619.49
		Feed cost (₹)	22.4	21.21	21.29	20.07
		Total feed cost (₹)	62.75	56.09	56.66	54.27
		Other expenditure*** (₹)	10	10	10	10
Total production cost*		(₹)	102.75	96.09	96.66	94.27
Live wt at 90 days of age		(g)	901.67	867	932.22	929.63
Gross return **		(₹/bird)	270.5	260.1	279.67	278.89
Profit		(₹/bird)	167.76	164.01	183.01	184.62

* Production cost includes chick cost and total feed cost only; ** Birds sold @ ₹300/kg live weight; ***Other expenditure includes supplementation cost and miscellaneous expenditure.

Dry matter and crude fibre digestibilities were significantly ($P < 0.05$) higher in xylanase supplemented group. These results are in agreement with the findings of Mathlouthi

et al. (2002), Berwal *et al.* (2008), Nian *et al.* (2011) and Stefanello *et al.* (2016). Nian *et al.* (2011) added xylanase in corn/soy-based diet and found non-significant ($P < 0.05$) effect on hemicellulose digestibility. However, hemicellulose digestibility value was numerically increased by 6.6%.

The economics of feeding Kadaknath birds with beta-xylanase and cysteine protease enzyme are presented in table 5. The total cost of production in T₀, T₁, T₂ and T₃ was recorded as ₹ 102.75, 96.09, 96.66 and 94.27, respectively whereas the profit per bird in T₀, T₁, T₂ and T₃ were observed as ₹ 167.76, 164.01, 183.01 and 184.62, respectively. The lower cost of production and higher profit per bird were recorded in both beta-xylanase and cysteine protease enzyme supplemented group. It might be attributed to compensation of nutrient digestibility of reduced energy and protein which is responsible for almost similar performance as in control group.

CONCLUSION

On the basis of the results of this study, it can be concluded that the growth performance was similar in xylanase, protease and combination of both supplemented group with reducing energy and protein in comparison to control group and nutrient utilizations were improved in beta-xylanase and cysteine protease supplemented group in comparison to control group. Inclusion of enzyme supplementation in rations containing reduced ME and CP % as compare to control diet, improved economics. Combination of xylanase and protease was more profitable in rearing of Kadaknath birds. This experiment proves that the effect of beta-xylanase and cysteine protease are similar in Kadaknath birds as they are found in commercial broiler chicken.

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