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Effect of Cysteine Protease in Diet to Reduce Soybean Meal Without Affecting Performance of Kadaknath Birds in India

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ABSTRACT

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. Indegenous breed "Kadaknath" is well known for delicious black flesh with special capabilities such as aphrodisiac properties, adaptability to local environment, resistance to certain diseases and meat quality. It has been neglected commercially because of its poor production potential. In an experiment of twelve weeks, one hundred Kadaknath chicks were randomly divided into two equal groups with five replicates, each replicate consisted of ten chicks. One of the two diets was supplemented with cysteine protease @ 250 gm per ton with 4% reduction of crude protein, lysine and methionine. The metabolizable energy was reduced at the level of 50 Kcal per Kg of diet to meet the requirement of the birds as per BIS (2007). Approximately min. 20 kg of soybean meal (45 % CP) was reduced in cysteine protease supplemented diet per ton. The body weight gain and FCR were statistically similar in both groups. The crude protein and crude fat digestibility were significantly ($P<0.05$) higher in cysteine protease supplemented group. Profitability was numerically higher in cysteine protease group due to reduction of soybean meal in diet. In the above study, it was concluded that cysteine protease is very economic enzyme to improve performance of birds and fulfil scarcity of high protein ingredients like soybean meal.

Keywords: Cysteine protease, Kadaknath, enzyme, soybean meal, profitability

Soybean meal dominates the market for protein supplements for poultry. There are a number of reasons for this, including its consistency in nutrient content, its ready availability year-round, and its high content of crude protein. Soybean meal is perhaps the only common protein supplement that is typically included in poultry rations with no limitation as to the quantity used. 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).

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). Indegenous breed "Kadaknath" is well known for

delicious black flesh with black beak, feathers, comb and shank in India. The bird is very popular due to its special capabilities such as aphrodisiac properties, adaptability to local environment, resistance to certain diseases, meat quality and many other criteria specific to breed type. It has been neglected commercially because of its poor production potential (Rao and Thomas, 1984). Efforts for attaining higher body weight in short duration is still a subject of research for this breed. 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 cysteine 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 one hundred straight run, day old Kadaknath chicks belonging to same hatch were weighed individually and randomly distributed into two 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. 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 0.250 kg/ton.

Cysteine protease enzyme (min. 32500 pu/kg) with reducing dietary ME 50 Kcal/kg and 4 % CP, lysine and methionine. The cost of all the experimental diets were worked out after considering the cost of ingredients, supplements and cost of enzyme supplementation. The average body weight gain, average feed intake, feed conversion ratio and efficiency index were recorded fortnightly. A metabolic trial was conducted during 6^{th} fortnight of growth period for analysed crude fibre and crude fat digestibility. Five birds per treatments were slaughtered after six fortnights of age to study the carcass yield. The data generated through the experimental period were subjected to statistical analysis by General Linear Models (GLM) procedure of the Completely Randomized Design to study the effect of treatment on various parameters (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The data regarding overall growth are presented in Table 2. The growth parameters include body weight, feed consumption, feed conversion ratio and efficiency index were not significantly different ($P > 0.05$) between treatments. However, numerically improved body weight, FCR and EI were recorded in cysteine protease enzyme supplemented group. It may be due to supplementation of single activity cysteine protease enzyme which compensate reduced level of protein and energy in the diets of T_1 in comparison to T_0. Similar results were found by Kocher *et al.,* 2003, Odetallah *et al.,* 2005, Wang *et al.,* 2006, Cowieson *et al.,* 2010 and Fru-Nji *et al.,* 2011 who also reported non-significantly ($P < 0.05$) different feed consumption in protease supplemented groups in comparison to control. Odetallah *et al.* (2005) also reported non-significant ($P < 0.05$) differences in feed consumption among the control and enzyme treatment groups.

Kocher *et al.* (2003) reported non-significant FCR in an experiment with 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 protease supplemented groups.

Odetallah *et al.* (2005) observed similarly 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.

Table 1: Composition and quantities of feed ingredients and additives kg per ton in the rations

Ingredients	T_0			T_1		
	Pre starter	**Starter**	**Finisher**	**Pre starter**	**Starter**	**Finisher**
Maize	568	575	613	608	615	653
Soybean meal	340	318	268	314	291	242
Calcite	6	6	6	6	6	6
Di calcium phosphate	4	4	5	4	4	5
Meat & Bone meal	50	50	50	50	50	50
Oil	19	35	46	5	21	32
DL-Methionine	1.8	2.1	2.1	1.4	1.7	1.7
L-Lysine	0.9	0.4	0	1.2	0.7	0.0
Soda Bicarbonate	1	1	1	1.00	1.00	1.00
Salt	2.5	2.5	2.5	2.50	2.50	2.50
Trace Minerals	1.25	1.25	1.25	1.25	1.25	1.25
Vitamin Premix	0.6	0.5	0.5	0.60	0.50	0.50
Acidifiers	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
Herbal choline	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
Betaine	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
Toxin Binder	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
Protease	0	0	0	0.25	0.25	0.25
TOTAL	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Chemical composition of rations						
Crude Protein (%)	22.96	21.94	20.06	21.76	20.72	18.68
ME (Mcal/Kg) *	3.00	3.1	3.2	2.88	2.95	3.05
Calorie protein ratio*	130.43	140.91	160	131.95	142.15	162.46
Crude Fibre (%)	3.75	3.63	3.45	3.66	3.61	3.43
Ether extract (%)	4.43	5.98	7.14	2.62	2.72	3.74
Amino acids						
Lysine* (%)	1.3	1.2	1.03	1.25	1.15	0.95
Methionine* (%)	0.53	0.55	0.52	0.49	0.51	0.48
Minerals						
Calcium* (%)	1	1	1	1	1	1
Avail. phosphorus* (%)	0.45	0.45	0.45	0.45	0.45	0.45

* Calculated values

Table 2: Overall growth of experimental birds

Treatment	T_0	T_1
Body weight (g)	901.67± 166.32	932.22± 65.94
Feed consumption (g)	2785.10± 348.75	2645.20± 157.67
FCR	3.18± 0.32	2.92± 0.23
EI	277.03±31.44	283.72±47.15

Note: Means with in column bearing different superscripts differ significantly (P < 0.05
Each value is a mean of five replicates

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 3.

Table 3: Average nutrient utilization (%) of experimental birds

Treatment	T_0	T_1
Dry matter	59.91±4.18	58.85±5.75
Crude protein	48.04^{b}±10.12	56.79^{a}±6.71
Crude fibre	30.89^{a}±10.10	22.28^{b}±8.89
Ether extract	51.65^{b}±11.45	72.49^{a}±12.20

Note: Means with in column bearing different superscripts differ significantly (P < 0.05)
Each value is a mean of five replicates.

The crude protein and crude fat digestibility was recorded significantly (P > 0.05) higher in cysteine protease enzyme supplemented group. However, crude fibre digestibility was observed significantly (P > 0.05) higher in control group. Total tract apparent retention (TTAR) of N increased significantly (P < 0.05) with protease, xylanase and amylase (PXA) supplementation versus the control group 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 (illeal) brought about by enzyme supplementation was increased 2.9% (absolute value, 3.6 % relative increase) in trial of Zanella *et al.* (1999).

The economics of feeding Kadaknath birds with cysteine protease enzyme is presented in table 4. The total cost of production in T_0 and T_1 were recorded as ₹ 102.75 and 96.66, respectively whereas the profit per bird in T_0 and T_1 were observed as ₹ 167.76 and 183.01, respectively. The lower cost of production and higher profit per bird were recorded in cysteine protease enzyme supplemented group.

Table 4: Economics of experimental birds

Treatments				T_0	T_1
Chick cost			(₹)	30	30
Feed intake and cost of feed/bird	0-2 weeks	Feed intake	(g)	136	138
		Feed cost	(₹)	22.45	21.33
	3-6 weeks	Feed intake	(g)	956.37	928.69
		Feed cost	(₹)	22.77	21.65
	7-12 weeks	Feed intake	(g)	1692.66	1578.48
		Feed cost	(₹)	22.4	21.29
Total feed cost			(₹)	62.75	56.66
Other expenditure***			(₹)	10	10
Total production cost*			(₹)	102.75	96.66
Live wt at 90 days of age			(g)	901.67	932.22
Gross return **			(₹/ bird)	270.5	279.67
Profit			(₹/ bird)	167.76	183.01
Profit			(%)	163.27	189.34

* Production cost includes chick cost and total feed cost only.

** Birds sold @ ₹ 300/kg live weight.

*** Other expenditure include supplementation cost and miscellaneous expenditure.

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 improved in cysteine protease supplemented group in comparison to control group. Nutrient utilization was also improved by inclusion of cysteine protease enzyme. Inclusion of enzyme supplementation in rations containing reduced CP % and ME (Kcal/kg) as compare to control diet, improved performance. Supplementation of cysteine protease was more profitable in rearing of Kadaknath birds. This experiment proves that the effect of cysteine protease is similar in Kadaknath birds as they are found in commercial broiler chicken.

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