Pak. J. Bot., 41(5): 2543-2550, 2009.

EFFECT OF ENZYME SUPPLEMENTATION OF BROILER DIETS CONTAINING VARYING LEVEL OF SUNFLOWER MEAL AND CRUDE FIBER

SHAHID RAZA[1], MUHAMMAD ASHRAF[2], TALAT NASEER PASHA[3] AND FAROOQ LATIF[4]

[1]*Quaid-i-Azam University, Islamabad*
[2]*National University of Science and Technology, Rawalpindi*
[3]*University of Veterinary and Animal Sciences, Lahore*
[4]*National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan*

Abstract

An experiment was conducted to evaluate the efficacy of multi enzyme in broiler diets containing varying levels of sunflower meal and crude fiber. Sixteen isonitrogenous experimental diets were prepared using 0, 5, 10 and 15% SFM and 4, 5, 6 and 7% CF, respectively with and without enzyme supplementation and fed to 480 day old broiler chicks. Data on feed consumption, weight gain, feed conversion ratio and dressing percentage was collected. Results showed significant differences ($p<0.05$) among different experimental diets for weight gain and feed conversion. The highest weight gain was observed in chicks fed on diet containing 10% SFM and 6% CF with Grindazym while the lowest weight gain was obtained on diet having 10% SFM and 6% CF without enzyme supplementation. Diet containing 15% SFM and 7% CF with NIBGE enzyme @1% of 2.5 Fold was found best on the other hand diet containing 5% SFM and 5% CF without enzyme supplementation had poorest feed conversion among all the experimental diets. Feed intake during the experimental period was not influenced ($p>0.05$) either by dietary SFM and CF level or enzyme supplementation. However, maximum feed was consumed by birds fed diet containing 5% SFM and 5% CF without enzyme supplementation. While the minimum feed intake was observed on diet formulated with 15% SFM and 7% CF without addition of any enzyme. Dressing percentage was calculated as carcass weight excluding skin including internal organs viz., heart, liver, gizzard and kidneys of the birds. No differences ($p>0.05$) were observed for dressing percentages on different experimental diets. Results from this experiment suggest that enzyme supplementation can improve nutritive value of high fiber broiler diets. Broiler chicks can grow faster and more efficiently on a diet containing fiber degrading enzymes than on a diet without enzymes.

Introduction

Sunflower meal (SFM) is a by product obtained after the extraction of oil from sunflower seeds. Crude protein content of SFM is 29 to 45%, depending on the dehulling and oil extraction process which has an inverse relation with its crude fiber (32 to 14%) contents (Mushtaq *et al*., 2006). It has been used in poultry diets as good source of protein (Senkoylu & Dale, 1999) but at very low inclusion level due to its high fiber and Lysine deficiency (Villamide & San Juan, 1998). The fiber content of SFM appears to be the most problematic factor concerning its use at high levels in chick diets (Senkoylu & Dale, 2006), which can be reduced partially by removing the testa through the decortication process (McDonald *et al*., 1977; Niazi *et al*., 1991), but in Pakistan, this method is not practiced.

Recent hike in the price of feed ingredients and reliance on vegetable protein sources has compelled nutritionists to explore any viable method to incorporate SFM in poultry diets at higher levels. Enzymes are added to facilitate the breakdown of larger molecular structures of the feed ingredients into smaller ones by their specific action and making these nutrients more readily available to the digestive system for better absorption. Successful attempts had been made to eliminate disadvantages of poorly digested feedstuffs by microbial cellulases and xylanases (Latif *et al*., 1996). Xylanases and cellulases prepared from *Chaetomium thermophile*, breakdown complex NSP linkages in sunflower meal and increase the caloric contents of feeds from 100-200 Kcal/kg feed (Rashid, 1999). Themophillic fungi are known to produce cellulases and xylanases (Latif *et al*., 1996). *Chaetomium thermophile*, a fungus, being a member of thermophilic family also have potential to produce cellutolytic enzymes (Singh *et al*., 1990). The enzymes extract obtained from (*Chaetomium thermophile*) fungal fermentation may degrade the cellulose and hemicellulose of SFM into their respective monomers.

It has been recognized that the disruption of cell wall matrix of SFM by exogenous microbial enzymes can lead to easy access of the endogenous proteolytic enzymes to digest the entrapped proteins (Choct & Kocher, 2000). Sorensen, (1996) has reported that supplementation of SFM based diets with microbial enzymes could increase the nutrient use of this product in layers and broilers. On the other hand El-Sherif *et al*., (1997) did not indicate any difference between the SFM without enzyme supplementation in broiler rations. Inconsistent results were reported by several authors regarding the use SFM with enzyme supplementation in poultry diets (Cowan *et al*., 1999; Kocher *et al*., 2000; Mushtaq *et al*., 2006; Aftab, 2009). An experiment was therefore, conducted to examine the effects of enzyme supplementation of broiler diets containing varying level of sunflower meal and crude fiber.

Materials and Methods

The experiment was conducted at Experimental Station, Department of Food and Nutrition, University of Veterinary and Animal Sciences, Lahore.

Birds, housing and management: Four hundred and eighty day old Hubbard broiler chicks of mix sexes were purchased from a commercial hatchery. All the experimental birds were initially weighed. The chicks were randomly divided into 48 experimental units of 10 chicks each.

The experimental room comprised of 48 pens and each pen housed 10 chicks. This broiler house was properly washed and disinfected by fumigation before the initiation of the experiment. All the experimental chicks were reared on floor throughout the experimental period of 6 weeks. The placement of each chick in the pen was also made at random. The temperature of the experimental room was maintained at 33±2°C during the first week of trial and then reduced by 3°C each week till it reached 24°C which was maintained for the rest of the period. Proper management practices like ventilation, sanitation etc. was practiced throughout the experimental period.

Experimental diets and chemical analysis: Sixteen isonitrogenous broiler starter diets were formulated according to the standards specifications of (Anon., 1994) for broilers

(Table 1). All the feed ingredients and enzymes were procured from local market and National Institute of Biology and Genetic Engineering (NIBGE), respectively. Experimental diets were as follows: Treatments A1, A2, A3 and A4 represent diets containing no sunflower meal with 4% crude fiber (CF) and enzyme supplementation @ 0, 1% of 2.5 fold, 1% of 5 fold and Grindazym, respectively. Treatments B1, B2, B3 and B4 represent diets containing 5% sunflower meal with 5% CF and enzyme supplementation @ 0, 1% of 2.5 fold, 1% of 5 fold and Grindazym, respectively. Treatments C1, C2, C3 and C4 represent diets containing 10% sunflower meal with 6% CF and enzyme supplementation @ 0, 1% of 2.5 fold, 1% of 5 fold and Grindazym, respectively. Treatments D1, D2, D3 and D4 represent diets containing 15 % sunflower meal with 7 % CF and enzyme supplementation @ 0, 1% of 2.5 fold, 1% of 5 fold and Grindazym, respectively. Each diet was randomly fed to three replicate pens for four weeks. Sixteen finisher diets were also prepared in the same manner (Table 2) and fed to birds for last two weeks. Chicks were fed ad libitum. Availability of fresh and clean drinking water was assured throughout the experimental period.

Sunflower meal was subjected to chemical analysis i.e., CP, CF, crude fat, ash and NFE according to A.O.A.C. (Anon., 2002) and fibre fractions according to Van Soest & Wine (1967). Optimum conditions for incubation as pH, temperature and enzyme to substrate ratio was 6.5, 56°C (Latif *et al*., 1996). Optimum incubation time was determined by taking 3g of Sunflower oil meal (SFOM) and 3 ml of enzyme along with optimum conditions for pH and temperature in 6 flask and then incubating them for different intervals of time viz., 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 hrs.

Data collection: Production performance (weight gain, feed intake and feed conversion) were measured for 6 weeks. Daily feed offered was recorded and the refused feed was weighed at the end of each week to determine weekly feed consumption. Weight gain was recorded weekly. Data on weight gain and feed intake was used to calculate feed conversion ratio (FCR) for starter and finisher phase separately. Mortality and general observations were also recorded during trial. At the end of the experiment, three birds from each experimental treatment were picked randomly and slaughtered to find out the dressing percentage. The comparative economics of different experimental diets was also calculated to determine the economic feasibility of using enzyme in high fiber broiler rations.

Statistical analysis: The experiment was conducted under completely randomized design with 4x4 factorial arrangements. The production performance data thus obtained were analyzed by analyses of variance (ANOVA) technique described by Steel *et al*., (1997). Mean values ± standard deviation (SD) are reported. Values were considered significant at $p \leq 0.05$. In case of significant differences Duncan multiple range test was employed to compare differences among means obtained during different treatments (Duncan, 1955).

Results

Data showing the effect of enzyme supplementation of broiler diets containing varying levels of sunflower meal and crude fiber on weight gain, feed intake and feed conversion is presented in Table 3. The highest weight gain (1696 g) was observed in chicks fed on diet C4 (10% SFM and 6% CF with Grindazym) while the lowest weight gain (1517.67 gm) was on diet C1 (10% SFM and 6% CF without enzyme supplementation).The statistical analysis of data revealed significant differences ($p<0.05$) among different experimental diets for weight gain.

Table1. Ingredient and nutrient composition of experimental broiler starter diets.

Ingredients	A	B	C	D
Maize	33.39	29.52	31.00	30.00
Wheat	16.00	16.00	15.00	05.00
Rice polish	06.00	08.00	08.00	10.66
Fish meal	06.00	05.98	04.63	05.00
Soybean meal	20.10	18.00	13.00	14.00
Cotton seed meal	04.36	05.80	05.00	05.58
Sunflower oil meal	0.00	5	10	15
Corn gluten 60%	07.00	05.92	06.00	07.00
Oil	02.95	02.30	02.50	02.00
Molasses	2	2	2	2
Lime stone	0.93	0.97	1.10	1.16
DCP	0.71	0.62	0.76	0.56
Premix	0.50	0.50	0.50	0.50
L-Lysine	--	--	0.08	0.10
Di-Methionine	0.06	0.07	0.08	0.06
L-Thrednine	--	--	--	--
Total	100	100	100	100
Calculated analysis				
Metabolizable energy (Kcal/kg)	3200	3200	3160	3160
Crude protein	23	23	23	23
Crude fibre (%)	4	5	6	7
Calcium (%)	1.00	1.00	1.00	1.00
Available phosphorus	0.45	0.45	0.45	0.45
Linoleic acid	3.96	4.60	4.78	5.00
Methionine	0.50	0.50	0.50	0.50
Lysine	1.10	1.10	1.10	1.10

Maximum feed (3238.33 g) was consumed by birds fed B1 diet containing 5% SFM and 5 % CF without enzyme supplementation while the minimum feed intake (3100 g) was observed on diet D1 which was formulated with 15% SFM and 7% CF without adding any enzyme (Table 3). However, feed intake during experimental period was not influenced ($p>0.05$) either by dietary SFM and CF level or enzyme supplementation. Dietary treatment affect ($p<0.05$) the feed conversion of broilers. Diet containing 15% SFM and 7% CF with NIBGE enzyme @1% of 2.5 Fold (D2) was found best and on the other hand diet B1 (containing 5% SFM and 5 CF without enzyme supplementation) had poorest feed conversion among all the experimental diets.

Dressing percentage was calculated as carcass weight without skin including internal organs viz., heart, liver, gizzard and kidneys of the birds. No differences ($p>0.05$) were observed for dressing percentages on different experimental diets.

Table 2. Ingredient and nutrient composition of experimental finisher diets.

Ingredients	A	B	C	D
Maize	46.33	41.10	37.00	38.86
Wheat	10	10	10	10
Rice polish	7	10.21	10	10
Fish meal	5	4.78	4.18	4.75
Soybean meal	12.96	10	13.89	8
Cotton seed meal	6.00	6.00	6.00	--
Sunflower oil meal	0	5	10	15
Corn gluten 60%	6.00	6.00	1.72	6.55
Oil	2.28	2.44	2.50	2.00
Molasses	2	2	2	2
Lime stone	1.02	1.06	1.10	1.00
DCP	0.94	0.89	0.89	0.43
Premix	0.50	0.50	0.50	0.50
L-Lysine	--	--	--	--
Di-Methionine	--	--	--	--
L-Thrednine	--	--	--	--
Total	100	100	100	100
Calculated analysis				
Metabolizable energy (Kcal/kg)	3170	3170	3170	3170
Crude protein	20	20	20	20
Crude fibre (%)	4	5	6	6.97
Calcium	1	1	1	0.90
Available phosphorus	0.45	0.45	0.45	0.45
Linoleic acid	3.39	3.99	5	5.2
Methionine	0.39	0.38	0.35	0.40
Lysine	0.92	0.88	0.94	0.85

Discussion

In the present study it was found that the different dietary treatments significantly affect ($p>0.05$) the weight gain and feed conversion (Table 3). Significant differences were observed with the incorporation of SFM in the non enzyme supplemented diets as compared to control having no SFM. However, with the increase in the level of SFM (5-15 %), no variation was noticed in weight gain of broilers. Enzyme supplementation improved ($p<0.05$) the weight gain of birds at every level of SFM. These findings suggest the role of enzyme in degrading crude fiber present in SFM based diets. Increased in the nutrient availability and metabolizeable energy may also be reasons for this improvements.

Table 3. Effect of enzyme supplementation and feed types on the broiler performance (0-6 weeks).

Dietary treatment				Production Performance		
Code	SFM	CF	Enzyme	Weight gain (g)	Feed intake (g)	Feed conversion (g/g)
A1	0	4	No	1548.33 ±1.67[f]	3223.33 ±6.67	2.08 ±0.006[b]
A2	0	4	N @1% of 2.5 Fold	1605.00 ±5.00[e]	3145.33 ±2.91	1.96 ±0.006[d]
A3	0	4	N @ 1% of 5 Fold	1783.33 ±3.28[b]	3181.33 ±8.11	1.89 ±0.006[ef]
A4	0	4	Grindazym	1691.67 ±1.67[ab]	3225.33 ±26.41	1.91 ±0.017[e]
B1	5	5	No	1526.33 ±3.18[g]	3238.33 ±41.06	2.12 ±0.024[a]
B2	5	5	N @1% of 2.5 Fold	1643.67 ±3.18[d]	3145.00 ±13.23	1.91 ±0.007[e]
B3	5	5	N @ 1% of 5 Fold	1669.00 ±10.69[c]	3183.33 ±27.28	1.91 ±0.010[e]
B4	5	5	Grindazym	1688.00 ±3.00[ab]	3203.33 ±30.75	1.90 ±0.017[ef]
C1	10	6	No	1517.67 ±1.33[g]	3125.00 ±22.55	2.06 ±0.15[bc]
C2	10	6	N @1% of 2.5 Fold	1665.00 ±2.89[c]	3131.67 ±6.01	1.88 ±0.003[ef]
C3	10	6	N @ 1% of 5 Fold	1684.00 ±5.57[ab]	3185.00 ±22.55	1.89 ±0.010[ef]
C4	10	6	Grindazym	1696.67 ±1.20[a]	3211.00 ±15.14	1.89 ±0.009[ef]
D1	15	7	No	1524.00 ±0.58[g]	3100.00 ±5.77	2.04 ±0.003[c]
D2	15	7	N @1% of 2.5 Fold	1686.67 ±3.33[ab]	3114.00 ±2.65	1.84 ±0.003[g]
D3	15	7	N @ 1% of 5 Fold	1682.00 ±3.00[b]	3189.33 ±10.33	1.90 ±0.006[ef]
D4	15	7	Grindazym	1692.67 ±1.45[ab]	3161.33 ±14.05	1.87 ±0.009[fg]

[a-g] Means within a column with no common superscript differ ($p<0.05$).
SFM = Sunflower Meal; CF = Crude fiber; N = NIBGE enzyme

These results are in line with the previous findings of Raj *et al*., (1988), Lee & Lee (1982) and Iqbal (1985) and Swain *et al*., (1996) who noticed improvement in the performance of broilers with the addition of multi enzyme in high sunflower diets. Sorensen (1996) also supported these findings as they reported that SFM contents could be increased from 5 to 16% in the diet without causing any adverse effect on production performance, provided optimum ME and lysine contents. These results were in accordance with the findings of Cowan *et al*., (1999) and Kocher *et al*., (2000) who reported an improvement in the nutrient digestibility due to enzyme addition at high inclusion of SFM.

The results of present study are in accordance with the findings of Marck & Splitek (1990) and Arora (1991) who concluded that cellulolytic enzymes when added to a high fibre diet of broiler chicks resulted in increased body weight. Similar findings regarding an increase in live weight gain of broiler with the addition of fungal enzymes complex were also observed by many workers (Brenes *et al*., 1993; Broz, 1994; Richter *et al*., 1992; Benabdejelil & Arbaoui, 1991 and Ermakova *et al*., 1992; Fuente *et al*., (1998). On the other hand, Abbas *et al*., (1998) had found that enzyme supplementation had non-significant influence on the feed gain ratio. But they had used commercial enzymes and the activity of commercial enzyme was not determined. That enzyme preparation might have lost its potency to degrade fibre or had very low activity. In contrast to our findings recent studies of Mushtaq *et al*., 2006 and Aftab (2009) revealed no significant of enzyme supplementation of SFM based diets for broilers. The variation in results might be due to differences in enzyme preparation used in these studies.

Feed intake of birds was independent of the inclusion of SFM in the experimental diets and their enzyme supplementation. The results also coincide with the findings of Abbas *et al*., (1998) and Naqvi (1996) who noticed non-significant difference in feed consumption among diets with or without supplementation of enzymes. In contrast to this study Marquardt *et al*., (1994) showed that enzyme supplementation resulted in increased feed consumption. The possible explanation for these differences may be the quality of the SFM used in experimental diets.

Conclusion

Results from this experiment suggest that enzyme supplementation may improve nutritive value of high fiber broiler diets. Broiler chicks can grow faster and more efficiently on a diet containing fiber degrading enzymes than on a diet without enzymes. Further research in this area is needed to confirm these findings and to elucidate the mechanisms which are responsible for the better performance of broiler chickens on diet with enzymes.

References

Abbas, W., S.H. Khan and M. Sarwar. 1998. Sunflower oil meal as a substitute for soyabean meal in broiler rations with or without multienzyme (Kemzyme). *Pak. Vet. J.,* 18(3): 124-129.

Aftab, U. 2009. Utilization of alternative protein meals with or without multiple-enzyme supplementation in broilers fed low-energy diets. *J. Appl. Poult. Res.,* 18: 292-296

Anonymous. 1994. Nutrient Requirements of Poultry. National Research Council. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Anonymous. 2002. *Official Methods of Analysis of the Association of Analytical Chemists*. Arlington Virginia, U.S.A.

Arrora, S.P., Y.P. Thakur and M.P. Narang. 1991. Influence of Novozyme on growth on chicks. *Poult. Abst.,* 18(9): 2213-2431.

Benabeljelil, K. and M.I. Arbaowi. 1991. The effect of dietary commercial enzyme preparation on performance of broilers. *Annales de Zootechni*., 40(4): 305-312.

Brenes, A., M. Smith, W. Guenter and R.R. Marquardt. 1993. Effect of enzyme supplementation on the performance and digestive tract size of broiler chicks fed wheat and barley based diets. *Poult. Sci.,* 72(9): 1731-1739.

Broz, J., P. Oldale, A.H. Perrin, G. Rychen and C.S. Nunes. 1994. Effect of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *Poult. Sci.,* 35: 273-280.

Choct, M. and A. Kocher. 2000. *Use of enzymes in non creal grain feedstuffs*. Proc. XXI World's Poult. Congr. WPSA, Montreal, Canada.

Cowan, W.D. 1999. Role of single activity xyalanase enzymes enzyme components in improving feed performance in wheat based poultry diets. *Agro Food Industry Hi-Tech.,* 4: 11-14.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics,* 11:1-42.

El-Sherif, K.H., D. Gerendai and T. Gippert. 1997. Complete substitution of sunflower meal for soybean meal with or without enzyme supplementation in broiler rations. *Arch. Geflu ¨gelkd.,* 61: 8-14.

Ermakova, V., Z. Petrina, B. Avdonin and M. Skuya. 1992. An enzyme premix. *Ptitsevodstvo,* 5: 12-14.

Fuente, J.M., P. Perez De Ayala, A. Flores and M.J. Villamide. 1998. Effect of strage and dietary enzyme on the metabolizable energy and digesta viscosity of barley-based diets for poultry. *Poult. Sci.,* 77: 90-97.

Iqbal, Z. 1985. *Effect of amino acids supplementation on the nutritive value of sunflower meal in broiler rations*. M.Sc. Thesis, Dept. Anim. Nutr. University of Agriculture Faisalabad.

Kocher, A., M. Choct, M.D. Porter and J. Broz. 2000. The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflowermeal. *Poult. Sci.,* 79: 1767-1774.

Latif, F., M.I. Rajoka and K.A. Malik. 1996. Thermostability of cellulases and xylanases from *Chaetomium thermophile*. Natl. Inst. Biotechnol. Genet. Engg. Faisalabad (Personal communication).

Lee, P.K. and M.C. Lee. 1982. Effect of feeding locally produced sunflower oil meal as protein supplement on the performance of the broiler chicks. *Jr. Taiwan Livestock Res.,* 15(2): 9-24.

Marek, J. and M. Splitek. 1990. The effect of cellulase from the mould *Trichoderma viride* on the performance of broiler chickens fed on high roughage mixtures. *Zivocisna Vyroba,* 35(12): 1069-1075.

Marquardt, R.R., P. Bores, W. Guenter and G. Corw. 1994. The nutritive value of barley, rye, wheat and corn for young chicks as affected by use of *Trichoderma reesei* enzyme preparation. *Anim. Feed. Sci.and Tech.,* 45: 363-378.

McDonald, P., R.A. Edwards and J.F.D. Greenhalgh. 1977. *Animal nutrition. 3rd ed*. The English Language Book Society. Hong Kong.

Mushtaq, T., M. Sarwar, G. Ahmad, M.U. Nisa and A. Jamil. 2006. The influence of exogenous multienzyme preparation and graded levels of digestible lysine in sunflower meal-based diets on the performance of young broiler chicks two weeks posthatching. *Poult. Sci.,* 85: 2180-2185.

Naqvi, L. 1996. *Bioavailability of metabolizable energy from rations as influenced by enzyme supplemented in broiler*. M.Sc. Thesis, Dept. Anim. Nutr. University of Agriculture Faisalabad.

Niazi, A.H.K., T. Kausar, F.H. Shah and W. Ishaque. 1991. Effect of reducing crude fibre content on the nutritive value of sunflower meal. *Pak. J. Sci. Ind. Res.,* 34(9): 321-323.

Raj, A.G., V.R. Sadagopan and P.V. Rao. 1977. A note on the chemical composition and nutritive value of sunflower seed meal. *Ind. Poult. Gazette,* 61(4): 130-134.

Senkoylu, N. and N. Dale. 1999. Sunflower meal in poultry diets: A review. *World's Poult. Sci., J.* 55: 153-174.

Senkoylu, N. and N. Dale. 2006. Nutritional evaluation of a high-oil sunflower meal in broiler starter diets. *J. Appl. Poult. Res.,* 15: 40-47.

Singh, A., R. Goel and B.N. Johri. 1990. Production of cellulolytic enzymes by immobilized *Sporotrichum thermophile. Enzyme Microb. Technol.,* 12(6): 464-468.

Sorensen, P. 1996. Sunflower + enzymes = soybean? *Feed Int.*, 12: 24-28.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd ed. McGraw Hill Book Co. Inc., New York.

Swain, B.K., T.S. Johri, A.K. Shrivastav, and S. Majumdar. 1996. Performance of broilers fed on high or low fibre diets supplemented with digestive enzymes. *J. Appl. Anim. Res.,* 10: 95-102.

Van Soest, P.J. and R.H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. *J. Assoc. Anal. Chem.,* 50: 50-55.

Villamide, M.J. and L.D. San Juan. 1998. Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. *Poult. Sci.,* 77: 1884-1892.

(Received for publication 27 March 2009)