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ENZYMIC DETOXIFIED MUSTARD SEED MEAL (DMSM) AS A SUBSTITUTE FOR VEGETABLE AND ANIMAL PROTEIN CONCENTRATES

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Abstract. Three experiments of 8 weeks each were conducted on day old PIA-Shaver Chicks. Enzymic detoxified mustard seed meal (DMSM) was used as a substitute of animal as well as vegetable proteins. These experiments showed that detoxified mustard seed meal could replace 100% of the sesame-seed meal; 66% of the blood meal; 33% of the fish meal, and 33% of blood meal, and 100% sesame meal from poultry feed without affecting growth. Incorporation of DMSM in the ration improved weight gain, feed efficiency, feed cost per kg of weight gain.

Poultry is one of the easy, short duration and efficient converters of low quality protein to proteins of high biological value. But efficiency of poultry production depends on feed cost which accounts for 60 to 70% of the total production expenditure.[1]

High price and scarcity of feed ingredients, particularly of proteinous nature, i.e. sesame seed cake, blood and fish meals, have created a difficult situation for the feed manufacturers and the poultry farmers. This appears to be the reason for utilization of only 26% of the available hatching capacity and necessitates exploration of the unconventional sources of good quality protein.

Rape and mustard seeds are abundently grown (401,000 metric tons/ year) in Pakistan as oil seed crops.[2] In addition to their importance as oil seeds, these can also be used as a potential source of good quality protein. The pressed cake left after oil extraction, contains 35 to 40% protein, with a well balanced amino-acid pattern comparable with that of Soya and other good quality proteins.[3-4] At present, a small portion of the seed cake is used in the rations of ruminants. But the ruminants are not as efficient converters of protein as poultry. However, nutritional qualities of mustard/rape cakes for poultry is imparied by the presence of toxic substances. These toxic substances are the split products of glucosinolates,[5] which yield isothiocyanate and oxazolidinethione upon enzymic hydrolysis. The isothiocyanate causes damage to skin, soft tissues and mucous membranes, while the latter has been linked with enlargement of thyroid glands.[6-8]

Toxic effects of rapeseed meal in chick rations were observed by Pettit *et al.*[9] Vogt *et al.*[10] recommended that the rapeseed meal content of broiler ration should not exceed 12%.

Feeding trials on rats indicated that mustard seeds (B. juncea) could be detoxified by removal of allylisothiocyanate, by steam stripping.[11] Detoxification of oil seeds and cake had been carried out by a number of workers.[12-21] Detoxified mustard seed cake was reported to be of high nutritive value by Shah *et al.*[22]

The aim of the experiment was to compare detoxified mustard seed meal as a substitute for sesame-seed cake, fish or blood meals. The mustard seed was detoxified by action of the endogenous enzyme myrosinase and by steam stripping.

Material and Method

Preparation and Analysis of Rations. All the feed ingredients were purchased from the local market except detoxified mustard seed meal (DMSM) which was prepared in these laboratories. Individual ingredients and the prepared rations were analysed for crude protein, crude fibre and total ash contents as reported earlier.[23] Volatile allylisothiocyanate was estimated by the method of Wetter.[24]

Detoxification of Mustard Seed Cake. Mustard Seed cake was detoxified according to the method of Shah *et al.*[22]

Experimental Birds. One hundred, day-old PIA-Shaver broiler chicks were used in the experiments. The chicks were weighed, wing banded and randomly divided into groups of 10 birds each. Two replicates of each feed treatment were set up. The chicks were raised in wire mesh cages with galvanized tin sheets on deep litre system. The temperature of the experimental room was maintained at $32 \pm 2^{\circ}$ in the first week and in the subsequent weeks it was lowered by 3°/week until it reached 26°. Composition of different experimental

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TABLE 1. REPLACEMENT OF DIFFERENT LEVELS OF SESAME MEAL BY DETOXIFIED MUSTARD SEED MEAL (DMSM).

Sr. No.	Ingredients	Control	50% Sesame replaced by DMSM	75% Sesame meal replaced by DMSM	100% Sesame replaced by DMSM
1.	Maize	46.00	46.00	46.00	46.00
2.	Jawar	12.00	12.00	12.00	12.00
3.	Wheat bran	8.00	7.00	6.00	5.00
4.	Rice polishing	4.00	4.00	4.00	4.00
5.	Rice husk				
6.	Corn gluten meal	3.00	4.00	4.00	4.00
7.	Sesame meal	8.00	3.00	2.00	-
8.	Enzymic detoxified mustard seed meal	-	5.00	7.50	10.00
9.	Guar meal	3.00	3.00	3.50	4.00
10	Blood meal	6.00	6.00	6.00	6.00
11.	Fish meal	6.00	6.00	6.00	6.00
12.	Molasses	2.00	2.00	2.00	2.00
13.	Bond meal	1.00	1.00	1.00	1.00
14.	Vitamin & Mineral Premix	0.50	0.50	0.50	0.50
15.	Lime stone	0.25	0.25	0.25	0.25
16.	Salt (NaCl)	0.25	0.25	0.25	0.25
	Total	100.00	100.00	100.00	100.00
Crude protein (analysed)%		20.75	21.35	21.24	22.10
Metabolizable energy (calculated)		1309	1303	1307	1307
Ca%		1.1949	1.1194	1.097	1.12
P %		0.85	0.79	0.79	0.79
Allyl isothiocyanate		-	Traces	Traces	Traces
Crude fibre %		2.5	2.6	2.6	2.58

TABLE 2. REPLACEMENT OF BLOOD AND FISH MEAL BY DETOXIFIED MUSTARD SEED MEAL (DMSM).

Sr. No.	Ingredients	Control	Different levels of blood meal replaced by DMSM			Different levels of fish meal replaced by DMSM			100% fish and 100% blood meal replaced by DMSM
			33%	66%	100%	33%	66%	100%	
1.	Maize	45.00	45.00	45.00	45.00	45.00	45.00	45.00	44.00
2.	Jawar	14.00	14.00	14.00	14.00	13.50	12.00	12.00	12.00
3.	Wheat bran	6.00	6.00	6.00	6.00	6.00	6.00	6.00	3.00
4.	Rice polishing	2.00	2.00	2.00	2.00	1.00	1.00	1.00	1.00
5.	Rice husk	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6.	Corn gluten meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
7.	Sesame cake	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
8.	Guar meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
9.	Blood meal	6.00	4.00	2.00	6.00	6.00	6.00	6.00	–
10.	Fish meal	6.00	6.00	6.00	6.00	4.00	2.00	–	–
11.	Detoxified mustard seed meal (DMSM)		2.00	4.00	6.00	2.50	5.00	7.00	12.00
12.	Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13.	Lime stone	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
14.	Vitamin & mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
15.	Molasses	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
16.	Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
	Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition									
Crude protein (analysed)		21.6	21.00	21.5	21.5	21.42	21.0	21.4	21.5
M Energy/kg		2860	2856	2860	2861	2848	2850	2865	2845

TABLE 3. REPLACEMENT OF FISH, BLOOD AND SESAME MEAL BY ENZYMIC DETOXIFIED MUSTARD SEED MEAL (DMSM)

Sr. No.	Ingredients	control	33% fish+33% blood replaced by DMSM	33% fish+66% blood replaced by DMSM	33% fish, 33% blood 1000% sesame replaced by DMSM	33% fish 66% blood 100% sesame	40% fish	50% fish
1.	Maize	45.00	45.00	45.00	45.00	45.00	45.00	45.00
2.	Jawar	12.00	10.00	12.00	12.00	12.00	12.00	12.00
3.	Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00	4.00
4.	Rice polishing	2.00	2.00	2.00	2.00	2.00	2.00	2.00
5.	Rice husk	1.00	–	–	–	–	–	0.25
6.	Corn gluten meal	5.00	5.50	5.50	5.50	5.50	5.50	5.50
7.	Sesame meal	10.00	10.00	10.00	–	–	10.00	10.00
8.	Fish meal	6.00	4.00	4.00	4.00	4.00	3.00	3.00
9.	Blood meal	6.00	4.00	2.00	4.00	2.00	6.00	6.00
10.	Molasses	2.00	2.00	2.00	2.00	2.00	2.00	2.00
11.	Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12.	Lime stone	0.25	0.25	0.25	0.25	0.25	0.25	0.25
13.	Vitamin & mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50
14.	Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
15.	Detoxified mustard seed Meal (DMSM)	–	4.50	6.50	14.50	16.50	3.00	3.75
16.	Guar meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
17.	Total L :	100.00	100.00	100.00	100.00	100.00	100.00	100.00

*Each kg of vitamin-mineral premix contained:- Vit. A. 1980000 I. U., Vit D_3 296000 I.U., Vit. E 2200 I.U., Vit. K 0.44 g, Vit. B_1 0.22 g; Vit. B_2 0.99 g; Vit. B_6 0.22 g; Vit. B_{12} 1.76 μg; Folic acid 0.11 g; Nicotinamide 4.4 g; Ca-pantothenate 1.32 g; choline chloride 66 g, Fe 4.84 g; Zn 11.22 g; Iodine 0.22 g; Cu 0.462 g; Cobalt 0.022 g.

rations used in first, second and third experiments is shown in Table 1, 2, and 3, respectively. All the rations were isonitrogenous and isocaloric within each experiment. Water and experimental rations were provided ad-libitum in mash form with continuous light.

Each cage was considered as a unit and the following observations were taken during the study: (1) Initial body weight, (2) Weekly weight/chick, (3) Weekly feed consumed/chick, (4) Mortality, (5) Final weight/chick, (6) Dressing percentage (skin removed).

All the chicks were slaughtered after 56 days and the dressing percentage was calculated. The data thus collected were subjected to statistical analysis, using analysis of variance based on completely randomized design[26] and the difference in mean values were tested by Duncan's Multiple Range Test (P 1,5).

Results and Discussion

The average weight gain of different groups of chicks reared on different experimental rations, for a period of eight weeks, is shown in Table 4. The results indicated that incorporation of enzymic detoxified mustard seed meal (DMSM) in the rations supported growth at all levels. The average rate of growth increased from 1510.5 g to 1810.5 g when 100% of the sesame meal was replaced by DMSM.

Sources of animal protein i.e. fish and blood meals, were also replaced by DMSM. Table 4 shows that replacement of 66% of the blood meal and upto 50% of fish meal did not affect the growth of the chicks. The data when subjected to statistical analysis, indicated a linear correlation between growth and replacement levels of sesame meal by DMSM, i.e. an increase in weight gain with an increase in the replacement levels of sesame meal by DMSM.

The differences in the nutritive value of the rations with higher levels of replacement of fish and blood meals by DMSM appears (Expt. 2nd and 3rd) to be due to the difference in the quality of the protein. These findings indicated that DMSM had better protein make up than sesame cake. However, it could not replace whole of the animal protein i.e. fish and blood meals. A comparison of the mean values by Duncan's Multiple range test

TABLE 4. AVERAGE WEIGHT GAIN, FEED EFFICIENCY, DRESSING PERCENTAGE AND ECONOMICS OF DIFFERENT EXPERIMENTAL RATIONS.

Rations with No.		Weight gain±S.E. g.	Feed efficiency ratio (per group)	Dressing percentage (without skin)	Feed cost per kg of Rations (Rs.)	Feed cost per kg of wt.gain (Rs.)	Mortality %
Experiment No. 1							
1.	Control	1510.5±29.00	2.65±0.03	67.0±2.0	1.19	3.15	Zero
2.	50% sesame meal replaced by enzymic detoxified mustard seed meal (DMSM)	1710.0±45.83	2.54±0.04	67.4±1.4	1.18	3.00	,,
3.	75% sesame meal replaced by (DMSM)	1753.5±37.70	2.48±0.03	68.0±1.1	1.07	2.58	,,
4.	100% sesame meal replaced by (DMSM)	1810.5±35.40	2.41±0.03	68.0±2.0	1.39	3.58	,,
Experiment No.2							
5.	Control	1590.0±51.00	2.57±0.05	68.8±0.8	1.34	3.44	,,
6.	33% Blood meal replaced by (DMSM)	1629.0±60.00	2.57±0.02	68.0±1.0	1.30	3.47	,,
7.	66% Blood meal replaced by (DMSM)	1583.0±78.30	2.67±0.01	65.0±0.8	1.26	4.57	,,
8.	100% Blood meal replaced by (DMSM)	1131.0±51.60	3.63±0.04	66.0±2.0	1.32	3.46	,,
9.	33% fish meal replaced by (DMSM)	1619.0±33.87	2.62±0.02	67.0±1.0	1.25	3.48	,,
10.	66% fish meal replaced by (DMSM)	1466.0±60.40	2.78±0.03	67.0±0.0	1.19	3.53	,,
11.	100% fish meal replaced by (DMSM)	1363.0±69.30	2.97±0.02	65.0±3.0	1.05	—	,,
12.	100% Blood & 100% Fish meal replaced by (DMSM)	913.0±46.70	4.13±0.08	65.0±3.0	1.05	4.34	,,
Experiment No. 3							
13.	Control	1319.0±63.00	2.78±0.04	61.38±0.48	1.82	5.06	,,
14.	33% fish replaced by (DMSM) & 33% blood	1400.5±67.30	2.63±0.05	60.00±0.85	1.78	4.47	,,
15.	33% fish and 66% blood replaced by M.S.	1248.0±80.50	3.42±0.04	59.49±70.77	1.67	5.71	,,
16.	33% fish, 33% blood and 100% sesame replaced by (DMSM)	1388.0±42.40	2.68±0.03	61.35±0.08	1.65	4.42	,,
17.	33% fish, 66% blood and 100% sesame meal replaced by (DMSM)	1254.0±78.10	3.33±0.02	61.42±0.63	1.59	5.29	,,
18.	40% fish replaced by (DMSM)	1314±45.0	2.67±0.02	61.27±0.63	1.77	4.73	,,
19.	50% fish replaced by (DMSM)	1309.0±75.3	2.71±0.03	60.87±0.27	1.76	4.77	,,

(DMR) showed that ration R_4 was significantly different from all the other rations while rations R_2 and R_3 were not significantly different. Similarly R_6 was significantly different (P 0.01) from all the other rations except R_{10}, R_7 and R_5 which were non-significant. These differences can be attributed to the amino-acid assortment and their availability in the DMSM.

Replacement of 33% blood and 33% fish meals (R_{14}) or 33% blood, 33% fish and 100% sesame meals (R_{16}) by DMSM gave small but statistically (P 50.05) non-significant improvements in weight gain. These studies also indicated that the chicks reared on different experimental rations in which animal proteins were partially replaced by DMSM, neither showed any abnormality in thyroid glands, as was noted by Giovannetti[30], nor any toxic effects as reported by

Bell *et al.*[27] In fact the growth was enhanced when 100% sesame meal (R_4), or 33% fish, 66% of blood and 100% sesame meals (R_{17}) were replaced by DMSM. The improvement in the quality of the rations as a result of DMSM addition was similar to that reported by Vogt *et al.*[10] Detoxification improved protein quality and eliminated toxicity attributed to the presence of glucosinolates.[7] The results indicated that detoxification was achieved without major damage to the protein.

Feed Efficiency. Progressive increase in the feed efficiency with an increase in the amount of DMSM was observed when upto 100% of the sesame meal was replaced by DMSM. The feed efficiency of the rations in which upto 66% of blood meal (ration 6 & 7) or 33% of fish meal (ration 9) was replaced by DMSM was comparable with the control.

Replacement of 33% blood and 33% fish meal (R_{14}), 33% fish, 33% blood and 100% sesame meal (R_{16}), 40% fish meal (R_{18}) or 50% fish meal (R_{19}) by DMSM improved the efficiency of the feeds. Duncan's test shows that only R_{14} and R_{18} were significantly more efficient than control. The statistical results indicated significant difference in weight gain, feed efficiency ratio among the various experimental rations in all the experiments. Commparison of the mean values revealed that replacement of sesame meal with DMSM resulted in a significant increase in feed efficiency. Fish and blood meals could be replaced upto 50% and 66% respectively without affecting the quality of feed. Replacement of higher levels of animal proteins by DMSM reduced efficiency of feed utilization. Our results agree with the observation of Bell *et al.*[28]

Dressing percentage and Mortality. Mortality was zero percent in all the experimental rations which indicated that the toxic factor was almost completely removed. The dressing percentage was calculated after removing the skin so the fat under the skin was not calculated. However, when the data were statistically analysed, for economical evaluation it showed non-significant difference among experimental rations. The data also indicated a decrease in feed prices per unit weight gain when DMSM was incorporated in the rations. A decrease in feed cost from Rs.3.14 to 2.56/lb was observed when different levels of sesame meal was replaced, by DMSM. A further decrease in the feed cost was observed in case of rations from which animal proteins were replaced by DMSM (Table 4).

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