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DIFFUSION EXTRACTION OF TOXIC FACTOR FROM MUSTARD SEED

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A process is decribed for the diffusion extraction of glucosinolate (the precursor of a toxic factor) from three *Brassica juncea* varieties of RL-18, poorbi Raya, and S-9 seeds. Diffusion extraction resulted in the elimination of glucosinolate to undetectable limits. Minor changes in oil, fibre and ash contents were due to extraction of soluble fractions with ethanolic-NaOH. The detoxified mustard seed meal showed higher recovery of 82.5 to 92.3 % and 89.1 to 95.4 % on weight/weight and protein/protein basis. The preboiling of RL-18 seed prior to diffusion extraction improved the NPU and PER of mustard seed meal from 48.3 to 52.0 % and 1.61 to 1.70 % respectively. The detoxified mustard seed meal could be incorporated into poultry feed.

INTRODUCTION

A balanced diet is of prime importance for human health but a majority of the population in the under-developed countries does not get the food in required calories. In most of the Asian countries the increase in population rate is much higher than increase in food. The population of Pakistan has increased from 65.30 in 1972 to 91.88 million in 1984 [1] and is expected to reach 142.70 million by the turn of the centurey. Efforts are being made to check this spurt in population but the problem of food deficieny still remains.

Pakistan produces 289,000 metric tons of mustard and rape seed [2] annually which is anticipated to increase in view of the increasing demand for edible oils. The cake left after oil extraction of mustard and rapeseeds is a potential source of protein. It contains 35 to 40 % protein [3] which has a well balanced amino acid profile [4]. A fair amount of basic sulphur amino acids [5] makes it more suitable to complement cereals and many legumes which are deficient in these amino acids. The presence of antinutritive factors i.e. glucosinolates [6], high amounts of fibre [7] and phytic acid [8] make it unfit for the non-ruminants and unpalatable to the ruminants. At present a small proportion of the seekcake is used in the ration of the ruminants, while the rest is either exported or used as a fertilizer. Many attempts have been made for reduction or complete elimination of toxic factors from mustard and rape seed/cake [9 – 17].

The object of the present investigations was to develop a procedure which would completely inactivate myrosinase responsible for the hydrolysis of the glucosinolate i.e. sinigrin, to toxic products followed by diffusion extraction of glucosinolate from the intact mustard seeds accompanied by minor loss in protein content. The nutritive value of the detoxified product was evaluated by conducting feeding trials on rats.

MATERIALS AND METHODS

Poorbi Raya, RL-18 and S-9 varieties of mustard seeds (*Brassica juncea*) used during the present investigations were supplied by the Punjab Agriculture Research Institute, Faisalabad. The proximate composition of the treated and untreated seeds, kernels and kernel meal and different diets was determined by standard methods [18]. The hydrolytic product of glucosinolate in mustard seeds. i.e. volatile allyl-*iso*thiocyanate was determined by Wetter's method [19]. The kernels were separated from the crushed seeds by air classification as reported elsewhere [20].

The detoxification of mustard seeds was carried according to the diffusion extraction procedure of Bhatti and Sosulski [16]. A weighed quantity (20 g) of the mustard seeds was extracted in screw-cap polythene bottles with 10 volumes of ethanolic-NaOH (0.01 N NaOH and 50 % v/v ethanol, pH 12) for 4 hr with a change of solvent every 2 hr (2 x 2). The extraction was carried out in a shaking waterbath at 60°, followed by filtration. The extracted seeds were rinsed with distilled water and dried for 2 hr at 60°. The seeds were crushed and extracted with *n*-hexane to reduce the oil to minimum. The mustard seed meal so obtained was dried at room temperature.

To determine the loss of protein nitrogen on diffusion extraction, ethanolic NaOH extracts were pooled and reducd in volume on a rotary evaporator. An aliquot of the extract was precipitated by adding an equal volume of 10 %

w/v trichloroacetic acid (TCA). After being allowed to stand at room temperature for 30 min the precipitated proteins were removed by centrifugation (10,000 x g; 20 min). An aliquot of the supernatant was taken for the determination of total nitrogen [18]. For comparative studies, the mustard meal i.e. defatted kernels were detoxified by aqueous extraction [14] and enzymic treatment of Shah *et al.* [17] in which the proteins in the supernatant were coagulated by passing steam for 30 min. The coagulated proteins were separated by centrifugation at 3000 rpm and added to the enzymic treated meal.

Preparation of Diets. Detoxified mustard seed meals, mustard meals and casein were mixed with a semi-synthetic diet containing minerals, corn oil, corn starch and glucose in such a manner that all the diets were isonitrogenous, i.e. they contained 10 % protein. The control diet (diet No. 1) derived all its protein from casein whereas diets No. 2, 3, 4 and 5 were prepared by replacing casein by 19.4, 18.8, 20.7 and 20.3 g. of enzymic treated, aqueous extracted mustard meals, untreated or preboiled and alcoholic-NaOH extracted mustard seedmeals respectively. The detailed composition of the diets is given in Table 4.

Biological tests. 21-day-old albino rats (Sprague Dawley strain) were taken for the nutritive evaluation of experimental diets. The net protein utilization (NPU) of the isonitrogenous control and experimental diets (10 % protein) was determined according to the method of Miller and Bender [21]. The protein efficiency ratio (PER) was calculated by dividing the weight gain with the protein intake [22] during the experimental period.

RESULTS AND DISCUSSION

The proximate composition of three varieties of mustard seeds i.e. RL-18, Poorbi Raya and S-9, is shown in Table 1. The protein and oil contents varied from 27.3 to 28.8 % and 34.3 to 44.6 % whereas the fibre and ash contents ranged from 6.7 to 9.3 % and 4.0 to 4.9 % respectively. The amount of allyl*iso*thiocyanate (AIT) in RL-18, Poorbi Raya and S-9 seeds was 1.08, 0.93 and 0.74 % respectively. The alcoholic-NaOH diffusion extraction increased the protein of the treated seeds from 29.3 to 30.0 %. The minor changes in oil, fibre and ash contents during diffusion extraction were due to the loss of soluble fractions of seeds (Table 1). These results are in accordance with the findings of Bhatti [16] and Sosulki *et al.* [23]. Moreover, the diffusion extraction reduced the glucosinolate contents of the seeds to traces. The present procedure not only removed the glucosinolate, the precursor of toxic factors, from intact seeds but also from low molecular weight compounds such as pigments, sugars and amino acids that react to form melanoids [24], imparting a dark

Table 1. Effect of alcholic sodium hydroxide diffusion on proximate composition of mustard seeds (*Brassica juncea*)

Local name	Treatment	Moisture (%)	Protein (%)	D.P.E.* (%)	Allyl *iso*thiocyanate (%)	Fat (%)	Ash (%)	Fibre (%)	Recovery (%) W/w basis	Recovery (%) Protein basis
	Nil	5.4	27.3	–	1.08	39.7	4.9	6.7	–	–
	Alcoholic-NaOH diffusion	2.7	29.5	2.5	Traces	47.5	5.9	7.7	82.5	89.1
RL-18	(i) Water boiled									
	(ii) Alcoholic-NaOH diffusion	3.1	29.4	2.2	"	46.2	4.5	7.8	84.7	91.2
	Nil	4.6	27.4	–	0.93	34.3	4.0	9.1	–	–
	Alcoholic-NaOH diffusion	2.9	29.3	2.4	Traces	40.1	4.5	10.6	84.6	90.4
Poorbi	(i) Water boiled									
Raya	(ii) Alcoholic-NaOH diffusion	3.2	28.1	2.2	"	37.7	5.1	10.1	88.9	91.1
	Nil	3.5	28.8	–	0.74	44.6	4.8	9.3	–	–
	Alcoholic-NaOH diffusion	2.6	30.0	1.8	Traces	48.8	5.3	10.0	90.1	93.8
S-9	(i) Water boiled									
	(ii) Alcoholic-NaOH diffusion	3.3	29.8	1.5	Traces	47.0	5.2	9.8	92.3	95.8

Diffused protein in extract

Table 2. Proximate composition of alcoholic-NaOH diffused mustard seed meals

Local name	Treatment	Moisture (%)	Protein (%)	Allylisothiocyanate (%)	Ash (%)	Fibre (%)
	(i) Alcoholic-NaOH diffusion (ii) Solvent extraction	3.1	48.4	Traces	8.1	14.7
RL-18	(i) Water boiled (ii) Alcoholic-NaOH diffusion (iii) Solvent extraction	3.9	49.3	"	8.9	18.5
	(i) Alcoholic-NaOH diffusion (ii) Solvent extraction	3.5	50.1	Traces	6.9	19.1
Poorbi Raya	(i) Water boiled (ii) Alcoholic-NaOH diffusion (iii) Sovent extraction	4.4	49.5	"	7.5	21.5
	(i) Alcoholic-NaOH diffusion (ii) Solvent extraction	3.3	53.7	Traces	9.4	18.6
S-9	(i) Water boiled (ii) Alcoholic-NaOH diffusion (iii) Solvent extraction	2.9	55.5	"	9.2	19.9

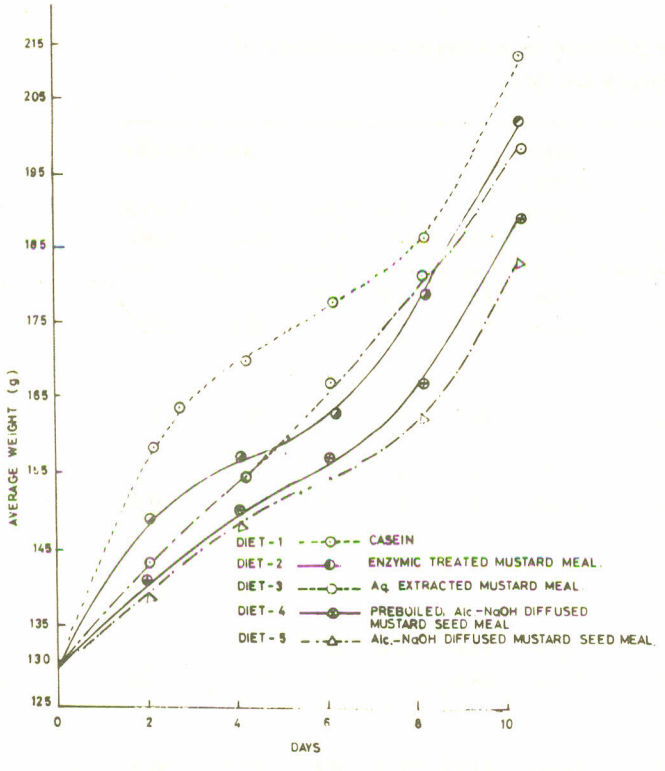

Fig. 1. Effect of incorporation of detoxified mustard seed meal and mustard meal on growth of rats.

brown colour to the meal. Diffusion extraction followed by extraction with *n*-hexane increased the protein, fibre and ash content of mustard seed meals (Table 2). Removal of the oil caused a corresponding increase in the respective seed components.

The group of four rats fed on control casein diet 1 and experimental diets 2-5 showed a weight gain of 82.0, 73.5, 74.7, 61.5 and 55.8 g respectively after ten days (Fig. 1), the maximum being in control casein diet followed by diets No. 3 and 2 supplemented with aqueous extracted and enzymic treated RL-18 meals. The better weight gain, higher NPU i.e. 67.0 and 64.0 % and PER i.e. 2.26 and 1.99 % (Table 5) of diets 2 and 3 containing enzymic treated and aqueous extracted RL-18 meals may be largely attributed to the complete removal of toxic AIT. This is in accordance with the findings of Lo *et al.* [25] and Anderson *et al.* [26] who have reported that detoxified rape flour has a PER equivalent to that of casein. The lower weight gain of rats, i.e. 61.5 and 55.8 g. fed on diet 4 and 5 and decrease in NPU, 52.0 and 48.3 % and PER, 1.70 and 1.65, of the respective diets (Table 5) might be due to reduced solubility and protein denaturation of proteins as a result of prolong heating at 60° for 4 hr the presence of AIT in bound form and high fibre content

Table 3. Composition of detoxified RL-18, mustard seed meal and mustard meal

Constituents %	Enzymic treated mustard meal	Aqueous extracted mustard meal	Water boiled, Alc.-NaOH diffused and extracted mustard seed meal	Alc-NaOH diffused and extracted mustard seed meal
Moisture	3.8	5.1	3.9	3.1
Protein	51.7	53.2	49.3	48.4
AIT*	Traces	Traces	Traces	Traces
Crude fibre	11.4	11.1	15.8	14.7
Ash	9.1	9.4	8.9	8.1
Fat	2.0	1.6	1.3	1.5
NEF**	22.0	19.6	20.8	24.2

* Allyl *iso*thiocyanate
** Nitrogen free extract

Table 4. Composition of standard and experimental diets

Ingredients	Casein (diet 1)	Enzymic treated mustard meal* (diet 2)	Aq. extracted mustard meal* (diet 3)	Alc.-NaOH diffused mustard seedmeal (diet 5)	Water boiled and alc.-NaOH diffused mustard seedmeel (diet 4)	Non-proteinous diet
Mustard meal	–	19.4	18.8	–	–	–
Mustard seedmeal	–	–	–	20.7	20.3	–
Glucose	10.0	10.0	10.0	10.0	10.0	10.0
Vitamins**	5.0	5.0	5.0	5.0	5.0	5.0
Minerals**	5.0	5.0	5.0	5.0	5.0	5.0
Corn oil	8.0	9.0	11.2	10.0	10.0	8.0
Corn starch	58.9	51.6	50.0	49.3	49.7	70.0
Cellulose	2.0	–	–	–	–	2.0
Casein	11.1	–	–	–	–	–
	100.0	100.0	100.0	100.0	100.0	100.0

*defatted and detoxified kernel
** Oser (1964) [28]

Table 5. NPU and PER of detoxified RL-18 mustard seed meal and mustard meal

Diet No.	Protein source	Protein intake (g)	Weight gain (g)	NPU (%)	PER
1.	Casein	33.12	82.0	75.0	2.47
2.	Enzymic treated mustard meal	32.50	73.5	67.0	2.26
3.	Aqueous extracted mustard meal	37.50	74.7	64.0	1.99
4.	Water boiled, ethanolic-NaOH diffused mustard seedmeal	36.25	61.5	52.0	1.70
5.	Ethanolic-NaOH diffused mustard seedmeal	34.75	55.8	48.3	1.61

contributed by hull fraction of mustard seed meal. Ballester *et al.* [27] and Shah *et al.* [17] have reported that the presence of toxic factor producing glucosinolates in untreated rape and mustard seeds were the main cause of depression in growth rate and decreased NPU, i.e. 40.0 and 45.8 % respectively. This clearly showed that alcoholic-NaOH diffusion extraction procedure improved the NPU of RL-18 seed meal by 8 to 12 %.

A better weight gain by diet 4 than diet 5 indicated that preboiling of seeds (for inactivation of myrosinase) followed by diffusion extraction of glucosinolate not only decreased protein losses in the extract from 2.5 to 2.2 % (Table 1) but also improved the NPU and PER of RL-18 seed meal from 48.3 to 52.0 % and 1.61 to 1.70 respectively (Table 5). Shah *et al.* [29] has reported that enzymic detoxified mustard seedmeal containing 39.25 % protein could replace 100 % of sesame meal, 66 % of blood meal and 33 % of fish meal from poultry feed without effecting growth. It can be safely concluded that mustard seedmeal detoxified by the present method can also be used in poultry feed as a substitute for animal proteins.

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