lipids. Kobayashi and Kanoh (1979) have reported that there is a marked increase in the deposition of fat in the heart with more circulating saturated fatty acids. The present study clearly indicates that feeding guar oil to rats leads to the development of atherorosclerosis.

During the present study it was observed that guar oil feeding caused the high fat deposition leading to liver enlargement that could be due to the presence of some toxic substances. The circulating lipids in guar oil fed rats had an accumulation in the heart, suggesting the development of atherosclerosis. Hence, the guar oil cannot be recommended for human consumption.

## ACKNOWLEDGMENT

We thank Dr. M. S. Naik, Head, Division of Biochemistry, IARI, New Delhi, and Dr. D. A. Dobholkar, Deputy Director, Shriram Institute for Industrial Research, Delhi, for their help and continued interest during the course of the present study.

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Received for review November 7, 1983. Revised manuscript received February 6, 1984. Accepted March 21, 1984.

# Improving the Biological Value of Guar Meal by Detoxification

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Guar seed meal is a byproduct of guar gum industry. It contains 45-55% of proteins along with several toxic substances like polyphenols, lignins, trypsin inhibitor, and saponins. It also contains some foul-smelling substances like organic acids, aldehydes, and cyanogens. The presence of the above toxic substances and foul-smelling compounds lowers the nutritive value of guar meal. Several solvent systems were tried for the detoxification of raw meal so as to make it good for consumption and to improve its biological value. Among several extracting systems, ethanol-HCl (100:1 v/v) was found to be suitable for removing most of the above toxic and foul-smelling components. The nutritional studies carried out on rats with such detoxified meal showed significant improvement in the biological value of the meal.

Guar seed meal, a byproduct of guar gum industry, contains 45-55% protein (Subramanian and Parpia, 1975) and 7% oil (Singh and Misra, 1980). A large portion of this meal remains unutilized owing to the presence of several toxic substances like polyphenols, lignins (Bajaj et al., 1978), trypsin inhibitor (Sumathi and Pattabiraman, 1976; Couch et al., 1966, 1967) and saponins (Arora and Joshi, 1980; Coxon and Wells, 1980) as well as some foul-smelling components, possibly organic acids, aldehydes, and cyanogens (Harborne, 1967). Presently 0.6 million ton of guar meal is available annually in India alone, and in years to come, the production is expected to be fairly high because of enormous demand of guar gum in world markets. Ingestion of guar meal is reported to have caused anorexia, diarrhoea, and decreased milk production in cattle (Natl. Dairy Res. Inst., 1960). Lower feed efficiency and depression in growth (Sathe and Bose, 1962) and mortality in animals (Kawatra et al., 1969) have been described as symptoms of guar meal toxicity. Attempts made for the detoxification of meal, earlier, have not been proven beneficial (Subramanian and Parpia, 1975). Toasted guar meal, however, has been reported to be a suitable replacement for up to half of the peanut cake as an ingredient poultry feed (Brahma and Siddiqui, 1978). This limited use of the meal will not keep pace with the increased production of guar. In the present paper the details of a procedure for the detoxification of guar meal and the improvement in the biological value are described. EXPERIMENTAL SECTION

Guar seed meal was purchased from a stockist (Delhi area) of guar gum industry in two lots. Endosperm splits were removed by sieving and finally by hand picking. Both the lots were mixed, ground to 80 mesh, and made moisture free. Lipids were extracted with ether by using the Soxhlet extraction procedure. The meal cake was dried and used for further experiments.

In order to arrive at a conclusion for the choice of an efficient detoxification method as finally applied on the meal, several other solvent(s)/extracting mediums were tried on a small quantity of the material. The main emphasis, during the course of these experiments, was placed on the minimum loss of the solids with concomitant removal of foul-smelling components. Since the condensed tannins contribute maximum to the toxic principle of guar, it was of interest to estimate them in the raw meal as well as in the processed meal. The tannins were estimated following the method of Burns (1971).

Extraction with Water. One-hundred grams of defatted guar meal flour was stirred with 400 mL of water in a Waring blender for 2 h at 1000 rpm. The contents were centrifuged at 4000 rpm for 15 min. The process was repeated 3 times for 1 h each. The residue was dried at 100 °C for 6 h. The water treatment failed to remove the foul smell but caused a loss of solids to the extent of 28%. It was able to remove tannins only to the extent of 32%.

Extraction with 70% Ethanol, Methanol, Ethanol, and Ethanol-HCl (100:1 v/v). Defatted guar meal was treated with these solvent systems, separately, following the procedure as described under Extraction with Water. Finally, the resultant cake was dried at 100 °C in the case of 70% ethanol and 60 °C in the other cases for 6 h. The estimated loss in flour weight was 30%, 9%, 6.5%, and 7.8%, respectively. To a certain extent, even after extraction, the foul smell persisted in all the dried residues except the one obtained after the ethanol-HCl solvent system. The removal of the condensed tannins, by the

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Table I. Biological Value (BV), True Digestibility (TD), Net Protein Utilization (NPU), and Utilizable Nitrogen (UN) of Guar Meal and Its Products after Detoxification

group no.	BVª	$\mathrm{TD}^b$	NPU	UN	
guar meal (raw)	e		-		
guar meal (defatted)	-	-	-	-	
detoxified guar meal	$\begin{array}{l} 62.16 \pm 5.82 \ (5); \\ p^d < 0.01 \end{array}$	$\begin{array}{l} 80.86 \pm 2.52 \ (5); \\ p < 0.05 \end{array}$	$50.75 \pm 5.86 (5);$ p < 0.01	$\begin{array}{c} 0.75 \pm 0.08 \ (5); \\ p < 0.01 \end{array}$	
protein from detoxified meal	$56.80 \pm 2.06 (5);  p < 0.001$	$91.47 \pm 2.28 (5);$ NS <sup>c</sup>	$52.00 \pm 2.46 (5);$ p < 0.001	$\begin{array}{c} 0.72 \pm 0.03 \ (5); \\ p < 0.01 \end{array}$	
skimmed milk (control)	$85.08 \pm 2.23$ (5)	$89.91 \pm 2.46 (5)$	$76.70 \pm 3.86 (5)$	$1.14 \pm 0.06$	

 $^{a}BV =$  corrected for metabolic and endogenous losses of nitrogen.  $^{b}TD =$  corrected for metabolic losses of nitrogen.  $^{c}NS =$  nonsignificant.  $^{d}p$  denotes the level of significance.  $^{e}All$  the rats died.

Table II.	Amino Acid Composition <sup>a</sup>	of Guar Mea	l and Its Products,	, FAO/WHO	<b>Provisional Patt</b>	tern (1973), and	Egg $(g/16 g of)$
<b>N</b> )							

	FAO/WHO		raw		protein of
	provisional		guar	detoxified	detoxified
amino acid	pattern (1973)	egg	meal	guar meal	meal
essential aa					
isoleucine	4.0	5.8	2.98	2.69	2.66
leucine	7.0	8.9	4.70	4.59	4.68
lysine	5.4	6.7	3.81	3.48	3.48
total aromatic aa	6.1	10.3			
phenylalanine	3.0	6.7	4.94	4.86	4.71
tyrosine	3.0	3.6	3.04	2.94	3.00
total sulfur aa	3.5	5.3			
cystine		3.0	2.68	2.67	2.68
methionine		2.3	1.13	0.89	0.84
threonine	4.0	5.1	3.96	3.45	3.40
tryptophan	1.0	1.5	1.29	0.95	0.99
valine	5.0	7.5	2.88	2.64	2.44
nonessential aa					
arginine	5.2	6.7	15.75	15.63	15.29
glycine	2.2	3.6	4.68	4.15	4.13
aspartic acid	7.7	10.4	9.75	9.64	9.66
serine	7.7	6.0	3.77	3.27	3.52
histidine	2.5	3.5	2.78	2.29	2.26
alanine	6.1	3.5	2.88	2.66	2.52
glutamic acid	14.7	25.2	23.49	22.98	22.98
proline	10.7		2.92	2.86	2.83

<sup>a</sup> Moisture- and fat-free basis.

above mediums, was found to be 48%, 85.4%, 80%, and 95.7%, respectively.

On the basis of the above experiments, it was inferred that the ethanol-HCl (100:1 v/v) solvent system could be an effective medium for the detoxification of the guar meal.

Details of Detoxification Method of Meal. Two kilograms of defatted guar meal was stirred with 8 L of the ethanol-HCl (100:1 v/v) solvent system in a Waring blender at 1000 rpm for 2 h. The dispersion was centifuged at 4000 rpm for 15 min. The residue was processed three times following the same procedure, as described above, except that the time for each treatment was kept to 1 h. The residue was finally stirred with 8 L of ethanol for 1 h to remove HCl. The meal was dried at 60 °C for 6 h. Ethanol was completely removed. The final moisture content at the time of preparation of the diet for feeding trials was 8.91%.

Isolation of Protein from Detoxified Meal. Half of the detoxified meal was dispersed in 10 volumes of water, and the pH was adjusted to 9.5 with 40% NaOH. The contents were well stirred and kept for 1 h at room temperature and then centrifuged at 4000 rpm for 15 min. The procedure was repeated once again to ensure maximum recovery. Both supernatants were pooled, and the pH was adjusted to 4.4 by HCl, in order to coagulate the proteins. The protein curd, thus formed, was removed and repeatedly washed, with four changes of water. It was finally lyophilized and weighed. Nitrogen estimation in all the samples was accomplished by using the conventional method of analysis (AOAC, 1965).

Amino Acid Analysis. The amino acid profile was determined by using a Technicon sequential multiple amino acid analyzer (Moore and Stein, 1963). Defatted samples containing 3–3.5 mg of nitrogen were hydrolyzed after evacuation in hydrolyzing tubes with 12 mL of 6 N Hcl (distilled) at  $110 \pm 1$  °C for 24 h. The contents were cooled and transferred to round-bottom flasks. The HCl was removed by flash evaporation at 50 °C under reduced pressure. The residue, after several washings with doubly distilled water, was finally taken up in citrate buffer (0.1 M), pH 2.0, and made to a known volume. An aliquot of the hydrolysate was used for determining the amino acid composition including cystine and methionine. The recovery of cystine under these conditions was  $90 \pm 2.0\%$ . Tryptophan was estimated following the method of Spies and Chambers (1949).

**Biological Evaluation Procedure.** Experiments were carried out by taking five groups, each containing five weanling rats (three males and two females), following the method of Mitchell and Carman (1926) as described by Lodha et al. (1976). The total period of 9 days consisted of preliminary feeding trials for 4 days followed by a 5-day balance period for the actual experiments. During preliminary feeding trials the rats were fed with the same diet that was given to them during the experimental period. The pooled feces and pooled urine for each rat were ana-

lyzed separately for nitrogen content by using the conventional Kjeldahl method (AOAC, 1965).

**Preparation of Diets.** The diets for biological experiments were prepared at a 10% protein level with powdered samples of raw, defatted, and detoxified guar meal as well as the protein isolated from detoxified meal. The composition of 100 g of diet was as follows: the test sample flour (calculated weight so as to give a 10% protein level), groundnut oil [calculated weight to give a 10% fat (containing 1 mg or 100 IU of vitamin E)], 4% mineral mixture (USP × VII4) composition as per Sikka et al. (1975), 5 g of glucose, and 5 g of complete vitamin mixture (Manna and Hauge, 1953) with 2 drops of adexolin containing vitamin A (12000 IU) and vitamin D<sub>2</sub> (IP, 2000 IU/g). The diets were fed orally daily. Skimmed milk was taken as the standard in the present experiment.

The biological value and related parameters were computed according to the method described by Chavan & Duggal (1978).

## RESULTS AND DISCUSSION

It was observed that the rats maintained on the raw and defatted guar meal diets died after the third day of their consumption, concluding thereby that the above diets were quite fatal to the experimental rats. It was, naturally, because of the toxic principles present in these diets. On the contrary, the rats kept on the detoxified meal and its isolated protein apparently maintained good health and survived throughout the course of experiment. This suggests that the ethanol-HCl solvent system used for the removal of foul-smelling and fatal substances could be taken up for the purpose of detoxification of guar meal. The suitability of the detoxification procedure may further be adjudged by the results (Table I) on the true digestibility (TD) of detoxified meal and its protein, which correspond well with the TD of the standard, skimmed milk. In addition, these findings may again be substantiated by the data presented in Table II, which exhibited a negligible loss in the contents of individual amino acids as a result of processing.

On viewing the data of the amino acid profile (Table II), it may be inferred that the quality of guar meal protein is very poor as it is deficient in various essential amino acids. However, the biological value (BV) and TD of the detoxified meal may be considered at par with those of some of the varieties of common pulses. The BV of the isolated protein was lower than that of the meal. This may probably be because of the fact that the detoxified meal originally contained some nutrients and crude fiber in addition to the amount of nutrients supplemented in the diet that helped in rapid absorption of the meal. Again, a significantly lower value of net protein utilization (NPU) and utilizable nitrogen (UN) (Table I) and close comparison of the essential amino acid pattern of these products with the FAO/WHO pattern and egg protein evidently indicate that protein as such may not play a major role in the building of the body, even after detoxification. Since the detoxified product is not at all fatal to the animals, it is thought that its supplementation with other diets could convert the meal into a more utilizable and useful dietary product.

#### ACKNOWLEDGMENT

We are thankful to Dr. M. S. Naik, Head of the Division, for providing facilities.

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Received for review May 31, 1983. Revised manuscript received March 21, 1984. Accepted April 1, 1984.