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Toxic and antigrowth effects of raw and processed field bean (*Dolichos lablab*) on albino rats†

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Abstract. Samples of freeze dried green field bean (*Dolichos lablab*) and dry mature bean, were subjected to the following processing methods—heat processing, extraction with 80% ethanol, hexane or dilute acid, protein isolation; and these samples were evaluated for growth promoting value and toxicity. Extraction with 80% ethanol or with dilute acid increased survival period of the animals; but these did not promote growth. Heat processing was essential to destroy antinutritional factors and promote growth. Extraction of the beans with 80% ethanol did not however alter the trypsin inhibitor or haemagglutinin activities. The protein isolate and acid-extracted residue which had low trypsin inhibitor and haemagglutinin activities, did not also promote growth. Thus the trypsin inhibtor and haemagglutinin activities did not completely account for the toxicity to albino rats. However, heat processing of ethanol extracted bean flour indicated that the beneficial effect of ethanol extraction was not apparent, once the samples were heat processed. Dry mature bean dhal was more toxic than the whole bean either dry or green. Supplementation of heat processed field bean with methionine and tryptophan promoted good growth of albino rats and significantly increased the protein efficiency ratio.

Keywords. Trypsin inhibitor; haemagglutinin; processing; nutritive value; *Dolichos lablab* (field bean).

Introduction

Grain legumes have been reported to contain a variety of anti-biological factors (Liener, 1962). Field bean (*Dolichos lablab*) both in tender green and mature dry stages is consumed after cooking in India and parts of South America. The raw bean does not support growth of young albino rats when fed as the sole source of protein in an otherwise adequate diet (Swaminathan, 1938; Hirwe and Magar 1953; Phadke and Sohonie, 1962b). Even after heat processing, it failed to support growth unless supplemented with all the essential amino acids (Phadke and Sohonie, 1962b). Hirwe and Magar (1953), however, observed that auto-

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Abbreviations used : protein efficiency ratio, PER.

claving of the bean and supplementation with methionine alone promoted very good growth and gave a protein efficiency ratio. (PER) of 3·0.

Raw mature field bean has been reported to contain trypsin inhibitor (Gaitonde and Sohonie, 1951), haemagglutinins (Hurpikar and Sohonie, 1961; Salgarkar and Sahonie, 1965a) and amylase inhibitor (Jaffe, 1973). Among these, the haemagglutinins showed the maximal adverse effects on the nutritional quality. However, it has been reported that haemagglutinins are only partly responsible for the growth retardation and toxic effects (Salgarkar and Sohonie, 1965b).

Phadke and Sahonie (1962a, b) have shown that the *in vitro* and *in vivo* digestibility of the field bean proteins are very low and that in addition to essential amino acid deficiency, these beans contain antinutritive factors. which are neither proteins nor peptides but that the factors are associated with the protein (Phadke and Sahonie, 1962c). Heat treatment has been shown to be beneficial, but selective extraction with solvents such as methanol or ethanol, or isolation of the protein was not beneficial (Phadke and Sohonie, 1962e).

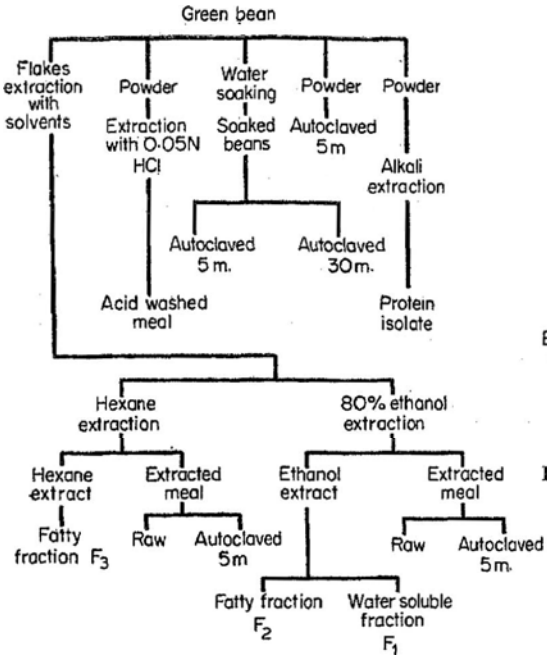
Very few studies have been reported on the fresh green beans. Hence it was of interest to study the effect of various types of processing such as autoclaving, selective extraction with solvents and isolation of proteins on the different toxic factors and to assess their relative roles on the nutritional value of the bean. In addition,the scope for improving the protein quality of the bean by amino acid supplementation was investigated.

Materials and methods

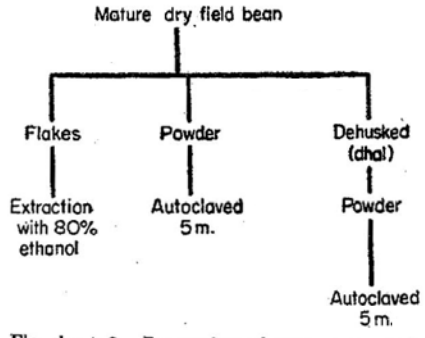
Green field beans procured from local market were cleaned, freeze-dried and stared in cold at 4° C. This is designated as green bean. Mature dry field bean was also procured locally. These were processed as shown in the flow-sheets 1 and 2.

In case of selective washing with 80% aqueous ethanol and hexane, the flaked beans were steeped in the respective solvent overnight and the extract drained. This was repeated four times to ensure thorough extraction of the solubles. The pooled aqueous ethanol extract was concentrated under vacuum (pressure of 35 mm mercury) at a temperature below 45° C and the concentrate obtained was fractionated with low boiling petroleum ether (b.p. range 40–60° C) to yield a water soluble fraction and a fatty fraction. The water soluble fraction was freeze-dried and designated as F_1. The fatty fraction, freed of the petroleum ether, was designated as F_2. The fat recovered from the hexane extract of the green bean flakes was designated as F_3 (flow sheet 1). In the case of acid extraction with 0·05 M HCl, the pH of the dispersion was 4·2. The subsequent washings were done with distilled water, the pH remaining the same even after washing. Acid extracted residue was freeze-dried. Autoclaved samples were dried in a current of hot air at 60° C. Protein isolate was prepared by aqueous extraction of the bean flour at. pH 10 and isoelectric precipitation of the protein at pH 4·6. The wet isolate was washed twice with distilled water and freeze-dried.

All the samples were powdered to. pass through 60 mesh and analysed for moisture and protein. The trypsin inhibitor activity in the samples was determined according to the procedure of Kakade *et al.* (1969) with minor modification;



Flowsheet 1. Processing of green field bean



Flowsheet 2. Processing of mature dry field bean

the inhibitor extract was prepared by dilute hydrochloric acid (0·05 N) extraction of the samples according to Borchers *et al.* (1947) using 1 to 10 ratio of bean powder to solvent. The haemagglutinin content of the samples was measured by the procedure of Liener and Hill (1953). The amylase inhibitor activity was measured by the procedure of Jaffe *et al.* (1973). The essential amino acid composition of green bean meal proteins was determined by standard microbiological assay procedure and chemical score calculated (W HO/FAO, 1965).

Growth studies on albino rats were carried out according to the procedure of Campbell (1963a). The raw and processed field bean powders were incorporated in the basal diet in place of corn starch to give 15% protein level rather than the conventional 10% protein level used in PER studies. Many vegetable proteins give better growth response at higher level of protein. Any antinutritive effects become apparent at higher levels of protein meals in the diet (Campbell, 1963b). The basal diet contained 10% hydrogenated fat, 10% cane sugar, 4% salt mixture (Hegsted *et al.,* 1941) and 1% vitaminised starch (Campbell, 1963a) and 75% corn starch. The control diet contained skim milk powder or casein to give 10% protein level.

Young, weanling Wistar strain 21–23-day rats wereused in all studies unless otherwise mentioned. The rats were housed individually in wire-bottomed cages. The diet and water were given separately and *ad libitum.* Shark liver oil was provided along with the diet as source of vitamins A and D. The duration of feeding was 4 weeks. Weekly gain in body weight of animals and the diet t

consumption were recorded. Any visible toxic symptoms or abnormality in the animal were also recorded.

A pair feeding study was carried out to compare the growth promoting value of aqueous, alcohol extracted bean (heat-processed) and unextracted bean (heat-processed). Fractions F_1 and F_2 obtained from 80% aqueous ethanol extract were fed through a stomach tube to 4–week old rats (F_1 500 mg/day and F_2 210 mg/day) maintained on a basal diet containing the ethanol-extracted bean meal as a source of protein. In another study, fatty fractions F_2 and F_3 were administered along with safflower oil to young rats (4-week old), kept on a basal diet containing the 80% ethanol-extracted and autoclaved field bean meal as source of protein. For comparison, one group was force-fed with equivalent quantity of safflower oil only. The control group was given only the basal diet.

The effect of methionine and tryptophan supplementation at optimum level to green bean, raw as well as processed bean was studied. Statistical design, grouping and analysis of data relating to all the animal feeding tests were carried out according to standard procedures (Snedcor and Cochran, 1961).

Results and discussion

Effect of processing on compositional aspects of field bean

(i) *Protein content* : The protein contents of the variously heat-processed samples of green bean were all comparable (27–28%). Dilute acid extracted sample had a slightly lower protein content of 22% (table 1) and the protein isolate had 88%. Mature dry field bean had a protein content of 23% and moisture content of 10·3%. On moisture-free basis, its protein content was comparable to that of green bean samples.

(ii) *Trypsin inhibitor content* : The trypsin inhibitor content of the 80% ethanol-extracted and hexane-extracted meals were not altered appreciably whereas in the acid-extracted meal and protein isolate, the levels were lowered considerably. Autoclaving under pressure was essential to destroy the trypsin inhibitor activity completely.

(iii) *Haemagglutinin content* : Again haemagglutinin content of green bean was not affected by 80% ethanol extraction or hexane extraction whereas it was lowered by acid extraction or isolation of protein. Autoclaving of the green bean powder was necessary to destroy the haemagglutinin activity completely.

(iv) *Amylase inhibitor activity* : This could not be detected either in green bean or mature dry bean.

(v) *Essential amino acid content* : The essential amino acid content of green field bean proteins is presented in table 2. The proteins are rich in lysine but markedly deficient in sulphur amino acids and tryptophan as shown by chemical score. For amino, acid supplementation studies at 10% protein level in the diet, 0·27% DL-methionine and 0·07% DL-tryptophan were optimal, based on the egg protein pattern (WHO, 1965).

Table 1. Trypsin inhibitor and hemagglutinin contents of and growth response of albino rats, fed with green and mature field beans, raw and after processing.

Sample	Protein content (N × 6·25) %	TIU[1] per mg protein in meal	HU[2] per mg protein in meal	Growth response		Mortality[3] in 4 weeks
				1 week	4 weeks	
Green bean	29	8·0	70·1	−8·9	..	8/8
Green bean 80% ethanol-extracted meal	28	7·8	71·5	−3·7	−5·8	0/8
Protein isolate	88	3·0	7·0	−6·4	..	6/8
Acid washed meal	22	1·1	5·6	−1·1	−2·7	0/9
Mature dry bean	23	21·0	177·8	−8·0	..	8/8
Green bean hexane-extracted meal	28	7·3	72·6	−11·3	..	10/10
Green bean hexane-extracted and autoclaved	29	0·0	0·1	..	..	0/10
Green bean 80% ethanol-extracted and autoclaved (5 min)	29	0·0	0·1	+4·2	+33*	0/10
Green bean, autoclaved (5 min)	29	0·0	0·0	+5·3	+35*	0/10

[1] TIU trypsin inhibitor units; [2] HU–Haemagglutinin units; [3] NO, of rats dead/total No.
* SE is ± 0·94.

Table 2. Essential amino acid composition of green (freeze-dried) field bean.

Amino acids	Green field bean	Whole egg[a]	Chemical score[a]
	g/16 g N		
Lysine	5·50	6·40	119
Methionine	0·80	3·10	33
Cystine*	0·51	2·40	
Phenylalanine	4·80	5·80	97
Tyrosine*	2·18	4·20	
Leucine	8·80	8·80	140
Isoleucine	4·40	6·60	92
Valine	5·81	7·30	110
Tryptophan	0·80	1·60	69
Threonine	3·50	5·10	95
Total	37·10	51·30	

[a] WHO/FAO (1965)

* Cystine and tyrosine are considered along with methionine and phenylalanine respectively while calculating the amino acid scores.

Effect of processing on the nutritional aspect of field bean

Raw green bean fed as a sole source of [protein did not support growth of rats (table 1) and the diet consumption in the group also being very poor. All the rats died within three weeks. The animals showed typical toxic symptoms like crouching appearance, forehead swelling, sluggishness and loss of fur mainly around the genitalia. Rats fed with ethanol-extracted meal survived the test period of 4 weeks, were active and looked healthy. The loss in weight during the first week was also much lower being 3·7 g as compared to 8·9 g for raw bean fed rats.

The results presented in table 1 show that in the raw state all the three samples (mature bean, dehusked dhal and green bean) caused loss in weight and mortality of rats. The diet consumption was very low. The animals in these 3 groups died in the first three weeks. The dehusked pulse was more toxic than the whole bean, either mature dry or green. This might be due to higher concentration of antinutritive factors in the dhal.

Rats fed on the protein isolate diet lost weight more than in the case of the ethanol-extracted meal diet and 6 rats out of 8 in this group died within the fourth week. This showed that the antinutritive factors of raw bean were not eliminated during protein isolation, even though a major part of trypsin inhibitor and haemagglutinin was removed, while in the case of ethanol-extracted meal, the two factors remained practically same as in the green bean. The above results indicate that the growth response of animals and the mortality cannot be correlated with either trypsin inhibitor activity or haemagglutinin alone. Confirmatory evidence for the presence of antinutritive factors in 80% ethanol solubles is provided by the feeding tests using the water soluble fraction F_1 and fatty fraction F_2 prepared from the extract. Data presented in table 3 show that these fractions caused mortality while the control group fed on the ethanol-extracted field bean residue as the sole source of protein in the diet, survived the test period of 4 weeks. Also, the fatty fractions F_2 from the ethanol extract as well as, F_3 from hexane extract were found to have growth depressing effects, when administered along with safflower oil to rats maintained on a basal diet containing ethanol-extracted and autoclaved green bean meal as the sole source of protein (table 4). Safflower oil by itself did not cause any adverse effects on growth and PER.

Heat treatment of the bean as well as the bean flour eliminated mortality in rats and the animals were healthy. The gain in body weights over 4 weeks (table 5) were 22·4 g for the flour, 17·4 g and 12·9g respectively for the soaked bean autoclaved for 5 and 30 min respectively. Autoclaving the bean powder for 5 min gave a better PER as compared to that of beans soaked and autoclaved for 5 min or 30 min, the values being 1·28, 1·06, and 0·87 respectively. However, in all cases the gain in weight and PER were less than in the control fed on skim milk powder diet (gain in weight 43·9g and PER 3·05) at the lower level of 10% protein level. The low growth rates and PER are presumably due to the deficiencies of essential amino acids in field bean proteins. These results demonstrate that heat processing is essential for inactivating the proteinaceous antinutritive factors such as trypsin inhibitor and haemagglutinin, thus, promoting growth in albino rats.

Table 3. Response of albino rats force-fed with fractions F_1 and F_2 of green field bean.

Fraction	Total amount force-fed (g)	Sex of the rat	Mortality: No. dead/total No.
Fraction F_1 (Water soluble)	4–8	♂	3/8
	6–12	♀	6/8
Fraction F_2 (Fatty fraction)	2·4–4·8	♂	8/8
	2·0–4·0	♀	8/8
Control	—	♂	0/8
		♀	0/8

Protein level in diet 16% (80% ethanol-extracted green bean meal as source of protein); eight rats per group. The animals were force-fed for four weeks.

Table 4. Growth response of rats force-fed with fatty fractions F_2 and F_3 of green field bean.

Fraction	Amount of fat force-fed in 4 weeks: Field bean (g)	Amount of fat force-fed in 4 weeks: Safflower oil (g)	Gain in wt (g) (Mean ± S.E.)		Protein efficiency ratio (Mean ± S.E.)	
Fraction F_2	5·0	3·1	22·6	±6·2	0·80	±0·08
Fractoin F_3	5·0	3·1	25·3		0·56	
Safflower oil	0	8·1	44·6		1·08	
Control	0	0	56·3		1·09	

Protein level in basal diet 15·9%; 8 female rats per group; initial body weight 63 g; duration 4 weeks.

Pair feeding studies were carried out using autoclaved green bean and ethanol-extracted meal. The average gains in weight for the unextracted and extracted meals were 35 and 33 g respectively, the values being not significantly different. This shows that the beneficial effects of ethanol extraction observed in the raw sample are not apparent when the major heat labile antigrowth factors are destroyed by autoclaving. The gain in weight of rats fed on unextracted autoclaved field bean meal is higher (35 g) in this study (table 1), as compared to that presented

Table 5 . Effect of heat treatment of green field bean on the PER of proteins.

Source of protein	Protein intake (g)	Gain in body wt. (g) (Mean ± S.E.)	Gain in wt./g protein intake (Mean ± S.E.)
Beans soaked and heat-processed for 5 min	16·0	17·4±2·35	1·06±0·09
Bean flour heat-processed for 5 min	17·8*	22·4±2·03	1·28±0·10
Beans soaked and heat-processed for 30 min	14·2	12·9±2·00	0·87±0·09
Skim milk powder	14·3	43·9±2·5	3·05±0·09

Protein level 16% except for skin milk powder (10%); 8 male rats/group; 33 g average initial body weight; duration 4 weeks.

*The data are for 7 rats only. This has been taken into consideration in statistical analysis

in table 5 (which is only 22·4 g). This might be due to the lowered diet intake in the latter experiment which was carried out in a different season.

As in the case of ethanol-extracted meal, acid washing also helps to eliminate mortality within the first four weeks, even though there is no growth promotion as seen in the data presented in table 1. The antigrowth factors like trypsin inhibitors and haemagglutinins were mostly eliminated by acid washing which shows that these two factors alone do not explain the antinutritive and toxic effects of raw bean, and that other factors may also be present.

The effect of amino acid supplementation of diets containing green bean raw, ethanol-extracted or heat-processed, with or without ethanol extraction is illustrated in figure 1; data on diet consumption and gain in body weight during 4 and 8 weeks period for only those groups giving positive growth are given in tables 6 and 7.

Animals fed on green bean raw, with or without amino acid supplementation, as well as those fed on ethanol-extracted meal lost weight (figure 1), the survival period in the case of the supplemented groups and the ethanol extracted group being longer (6 weeks, group E) as compared to the raw bean group (2 weeks, group A). When the ethanol-extracted green bean was supplemented (group F), the growth was positive but low and was comparable with that of heat-processed meal (group C); the growth pattern of the ethanol-extracted meal, heat-processed (group G) was also similar. The most striking growth response was seen when the heat-processed samples were supplemented. The ethanol-extracted meal after heat processing and amino acid supplementation (group H) gave an average gain in weight of 63·6gin four weeks. The unextracted meal after autoclaving and amino acid supplementation (group D) gave a lesser gain in weight of 51·5 g during the same period. The corresponding PER values of the two groups

Table 6. Effect of supplementation of essential amino acid to processed field bean meal diets on the growth response of albino rats

Source of protein	4 weeks						8 weeks			
	Diet consumed (g) (Mean ± SE)		Gain in wt (g) (Mean ± SE)		PER (Mean ± SE)		Diet consumed (g) Mean ± SE)		Gain in wt (g) (Mean ± SE)	
Heat-processed field bean	134·3		10·0		0·81		249		17·5	
80% ethanol-extracted meal + meth. (0·27%) + tryp. (0·07%)	110·8	± 6·6	6·2	± 1·5	0·62	± 0·1	206	± 10·7	13·2	± 7·7
Extracted meal, heat-processed	131·8		9·0		0·66		245		8·3	
Extracted meal, heat-processed + meth. (0·27%) + tryp. (0·07%)	249		63·6		2·45		514		107	
Diet C+ meth. 0·27%) + tryp. (0·07%)	230	± 7·7	51·5	± 4·8	2·11	± 0·15	503	±46	118	±7
Casein (control)	257		86·5		3·27		562		123	

10 male rats per group ; initial body wt 34 g ; duration 8 weeks. Protein level in diet 10% ; meth.—methionine ; tryp.—tryptophan.

Test of significance given in table 7.

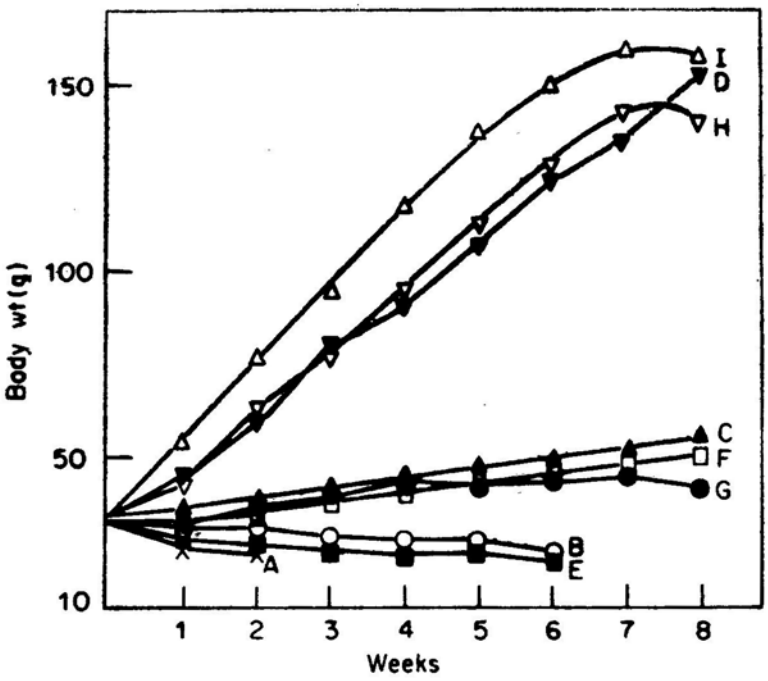


Figure 1. Effect of essential amino acid supplementation to raw and processed green field bean.

A, Green bean raw; B, Green bean raw + Dl-methionine + Dl-tryptophan; C, Autoclaved bean; D, Autoclaved bean + Dl-methioninine + Dl-tryptophan; E, 80% ethanol-extracted meal; F, 80% ethanol-extracted meal + Dl-methionine + Dl-tryptophan; G, 80% ethanol-extracted meal autoclaved; H, 80% ethanol-extracted meal + Dl-methionine + Dl-tryptophan; I, Casein.

Table 7. Tests of significance for the data in table 6 by Duncan's multiple range test.*

	4 weeks			8 weeks		
Group	F	G	C	F	G	C
Diet consumed	110·8	131·8	134·3	206	245	249
Group	F	G	C	G	F	C
Gain in weight	6·2	9·0	10·0	8·3	13·2	17·5
Group	F	G	C			
PER	0·62	0·66	0·81			
Group	D	H	I	D	H	I
Diet consumed	230	249	257	503	514	562
Group	D	H	I	H	D	I
Gain in weight	51·5	63·6	86·5	107	118	123
Group	D	H	I			
PER	2·11	2·45	3·27			

* Duncan (1955)

Table 8. Comparison of PER values of heat-treated samples of mature dry field bean, split dhal and green bean, freeze-dried.

Source of protein	Protein intake g.	Gain in body wt. g. (mean ± S.E.)	PER Gain in wt. per g. protein consumed (mean ± S.E.)
Dry bean (whole) powder heat-processed	29·0	34·3	1·19
Dry bean (dhal) heat-processed	17·5	14·8 ± 2·34	0·84 ± 0·085
Green bean powder heat-processed	28·0	36·2	1·28
Casein control	19·4	55·9	2·87

Protein level 15% except control (10%), 8 male rats/group; initial body weight 33 g; duration 4 weeks.

Table 9. Growth response of albino rats fed on mature dry field bean meal, extracted with 80% aqueous ethanol.

Source of protein	Loss in wt in 2 weeks (Mean ± S.E.)	Diet consumed in 2 weeks (Mean ± S.E.)	Mortality [No. dead/ total No.]
80% ethanol-extracted green bean meal	19·0 ±1·04	59·5 ±2·52	0/10
80% ethanol-extracted mature dry bean meal	20·3	69·3	10/10

8 male rats per group; initial body wt 66g; duration 4 weeks; protein level diet, 16%.

were 2·45 and 2·11 respectively. The control groups of rats fed on casein gave a gain in weight of 86·5 and a PER of 3·27. It may be noted that with the continuation of the feeding beyond 4 weeks, up to the 8th week, improved growth response was seen in the case of the bean diets supplemented with amino seeds with the final values comparing favourably with that of casein diet.

The growth response of rats fed on dry bean (whole) and dry bean dhal, both heat-processed, is presented in table 8. For comparison, green bean flour (heat-processed) was also included. Casein served as a control. Both dry and green bean gave better growth responses of 34 and 36 g respectively than the dry bean dhal which gave only 14·8 g in 4 weeks. Also the PER was better with whole

bean than dehusked dhal. However, the growth was very much lower than that of casein.

Ethanol extraction of mature bean was not beneficial unlike in case of green bean as evidenced by mortality pattern, though diet consumption or loss in weight were practically same for both dry and green beans (table 9).

Conclusions

The results reported in the present study while providing confirmatory evidence on the adverse growth and toxic effects of raw dry field bean (Phadke and Sohonie, 1962a, b, c, d, e) have brought out some new findings on the effects of alcohol extraction, acid extraction and heat treatment on the nutritive value of both dry and green bean. These are, (i) whole bean appears to have less pronounced toxicity than the dehusked dhal; (ii) ethanol extraction of green bean eliminated typical toxic symptoms and prolonged survival period of young rats. Similarly, acid extraction of green bean had beneficial effect; (iii) the antigrowth and toxic effects of the green bean are not due to only trypsin inhibitor and haemagglutinin; and (iv) heat treatment of both dry and green beans is essential for promoting growth in rats. However, even after heat treatment, the nutritional value of the protein is lower than that of casein presumably on account of amino acid deficiencies. This is borne out by the results of the supplementation studies with limiting amino acids methionine and tryptophan showing striking improvement in growth response and PER. The similarity of the results obtained in the case of the ethanol-extracted and unextracted meals after heat processing indicate the possibility that the anti-nutritional factors extracted by ethanol are also heat labile. Thus the antinutritive and toxic effects of raw green field bean appears to be many-fold.

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