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EFFECT OF SOME TOXIC FACTORS IN RAW BEANS ON ORGANS OF RATS

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SUMMARY

Autoclaved dry ground beans and ethanol extracted ground beans which contain two-third of the haemagglutinin, and one-quarter of the trypsin inhibitors with 80% of its original tannin were fed to rats for determination the Nitrogen digestibility. The toxic factors of beans killed all the animals before the completion of the 10-day period assay. Fecal DNA of animals were twice greater compared with casein and none-protein fed animals. Some cahnges were observed on organs weight(pancreas,spleen, liver, kidneys and adrenals) compared with controls.

INTRODUCTION

Phaseolus vulgaris was domesticated in Central America about 7,000 years ago(1).It was introduced in Europe in the sixteenth century, and since then it has become a common leguminous crop throughout most of the world.

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About 70 per cent of the protein in the human diet world-wide comes from vegetable sources, 30 per cent from animal sources,(2). Legumes are a particularly valuable protein supplement when the staple diet consists of root crops.

Raw legumes contain numerous toxins-trypsin and amylase inhibitors, lectins(haemagglutinins),(3)polyphenols, tannins, cyanogenetic glycosides,goitrogenic factors,lathyrogens, anti-vitamine factors, unidentified growth inhibitors, metalbinding costituents, estrogenic factors, and toxic amino acids. Most of these are destroyed by cooking, so that adequately cooked legumes are generally cosidered to be completely safe. With rare exceptions dried legumes are eaten only after cooking.(A report from the Public Health Laboratory Service (Brit.Med. J. 1268, ii 1976)stated that soaked, raw red beans eaten in Great Britain caused severe vomiting and diarrhoea).

Apparent and true digestibility in rats for nitrogen of cooked white kidney beans were 90% and 70% respectively(4).

Salman et al, (5) fed chickens autoclaved and unheated soybean meal and found growth depression and pancreatic enlargement in all chicks fed unheated diet.The pancreata were pale and white compared with pink in the control. A higher incidence of intestinal haemorrhages was also noticed. Higher levels of enzymes were reported and an increase of zymogen synthesis.

Haemagglutinins do not appear to cause a problem,since they are rapidly destroyed by heat; inadequately cooked legumes have bean stated to be toxic (6,7 and 8).

Jaffe (9) postulated that the action of the haemagglutinin was to combine with cells lining the intestinal wall, thus causing a non-specific interference with the absorption of nutrients. This effect of course will be refelected in the extent to which the protein is apparently digested. Korte (10) has observed that in a mixture of ground beans and ground cereal, prepared under the field conditions prevailing in Africa, the haemagglutinin was not always destroyed, and the cooked product produced diarrhoea and severe vomiting. An outbreak of massive poisoning after the consumption of partially cooked bean flakes has bean reported by Griebel (11).

The possible effect of toxins was examined by feeding incompletely cooked legumes and raw legumes after alcoholic extraction of flatulence factors (12).

Experiment:

White kidney beans (phoseolus vulgaris) Marmar from Isfahan(65-071-000212),which was prepared by Dr.Sarrafi (the School of Agriculture, Karadje, University of Tehran, IRAN), were ground and divided into two portions (A and B). Sample A was extracted twice with five volumes of ethanol (96%) and filtered. Then it was dried overnight at room temperature. Both samples(A and B) were canned and partly cooked by autoclaving for 30 minutes at 15 psi(121^{o}C) without the addition of water.The moisture content was 8-9% and under these conditions most of the haemagglutinin remains active. Four diets were prepared as follows:

A-Alcohol extracted and partly cooked beans 40% (i.e. approximately 10% protein).

B- Partly cooked beans 40%

C- Casein 10%

D- Non-protein.

The basic diet consisted of 80g corn oil,20g Sucrose, 40g vitamin mixture and 40g mineral mixture per kg, and made up to 1kg with corn starch.

20 weanling rats(Wistar Strain)were divided into four groups, each group containing five animals. The animals were fed for 10 days; food intake and weight gain were measured.

For determination of haemagglutinin, the methods of Liener(13) and Lis and Sharon(14) were used. That is,the haemagglutinin is calculated from absorbance measurements of the suspension of unsedimented rabbit red blood cells after a given time. Rabbit erythrocyte sediment at a rate which is proportional to the concentration of the haemagglutininating activity.

Results and Discussion:

Table 1 shows that autoclaving with low moisture content(8-9%), destroyed about one-third of the haemagglutinin, and three-quarters of the trypsin inhibitors.

The amounts left were sufficiently toxic to kill all the animals before the completion of the 10-day period for measuring digestibility. Table 2 shows the extremely low digestibility of nitrogen in these animals, namely about 30%, compared with the higher figure of 70% for true dry matter digestibility. The figures compared with 80% true nitrogen digestibility and 90% true dry matter digestibility in the completely cooked beans(4).

So it would seem that the toxins greatly increased metabolic nitrogen output.

These findings were confirmed in a duplicate experiment using heavier animals(approximately 45g instead of the 30g rats). These rats lived longer(averaqing 9 days, compared with 5-6 days), but showed the same low true nitrogen digestibility, namely 30% and true dry matter digestibility, namely 80%(Table 3).

Tables 4 and 5 show the various DNA contents of the faeces collected during the experiments .Although the DNA content of the faeces of the legume-fed rats was double that of the casein-fed rats, the DNA per mg faecal nitrogen was the same in all three groups in both experiments. For determination of DNA the method of Wannemacher et al(17), modified by Mohammadiha, (18)was used.

This suggests that the faeces contain a constant proportion of endogenous nitrogen irrespective of whether the diet is well "digested" (casein) or very poorly "digested" (toxic beans).

Organ Weights:

The mortality of the animals might have arisen partly as a result of starvation, since they ate less than the non-protein group, but the toxins probably played some part. On post-mortem, the intestines showed severe haemorrhage so it is likely that the toxins were partly responsible for the deaths.

Organ weights(Tables 6 and 7) are shown in absolute terms and per 100g of body weight. It is difficult to make any comparisons because, taking the pancreas weights as an example, they are smaller than the controls on

the experimental diets, but larger than non-protein groups, and larger than controls when calculated per 100g of body weight.

Since the non-protein rats were the same weight as the experimental groups and the pancreas weight/100g body weight was greater on legume diets, it appears that these pancreata were hypertrophied.

No conclusion can be drawn about liver and kidneys, but the spleens were very much smaller than either casein controls and non-protein rats in total weight and also in g/100g body weight.

The functions of the spleen are as vaguely understood today as they were centuries ago. The possible functions of the spleen are related to blood formation, storage and destruction, and the body's defence against infection(19) The small weight of spleen which has been observed in bean-fed rats can not easily be explained, as in most of the literature,reports of spleen enlargement are seen.In all the rats examined post-mortem,both those which died and those which were killed because they were dying,haemorrhage was observed in the intestine and other organs. So the small weight of the spleen might be due to contraction of the spleen to help to maintain the blood volume. This haemorrhage may be due to haemagglutinins as it was shown in Table 1 that even after dry cooking,beans contain a large amount (10,000 units/g) of haemagglutinin.

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Table 1

Haemagglutinin, tannin and trypsin inhibitor content of beans(Canellini)

Beans	Haemagglutinin units/gm (a)	Trypsin inhibitor TUI/mg (b)	Tannin O.D.(c)
Raw	15400	22.79	0.102
(A) autoclaved and ethanol extracted	9850	6.184	0.087
(B) autoclaved	10030	6.780	0.080

(a) (13)

(b) (15)

(c) (16)

Table 2

Weight gain, food intake, life time and true N and dry matter digestibilities

Groups of rats and diets	Initial Weight(g)	Life time (days)	Weight gain (g)	Food intake (g)	True N digestibility %	True dry matter digestibility
(A) 3 male and 2 female	29(+2.2)	6.4(+2)	-8(+2)	11.7(+1.5)	32.0(+1)	72.0(+4)
(B) 3 male and 2 female	30(±2.2)	5.2(±1.7)	-9(±1.5)	11.8(±2.0)	29.0(±5)	70.0(±6)
(C) 3 male and 2 female	30.4(±1.6)	-	+16.6(±4)	47.5(±6.5)	96.0(±0.9)	98.2(±0.56)
(D) 2 male and 3 female	31(±2.5)	-	- 7.8(±1.5)	25.5(±2.5)	-	-

Table 3
Weight gain, food intake, life time and true N and dry matter digestibility of rats on different diets

Groups of rats and diets	Initial weight	Life time (days)	Weight gain (g)	Food intake (g)	True N digestibility %	True dry digestibility %
(A) Alcohol Ext. beans 40%	47.7(±4.5)	9.2(±1.7)	-12.7(±2.1)	17.5(±8.2)	28.6(±0.7)	77.4(±2.3)
(B) Beans 40%	46.7(±3.2)	9.3(±1.3)	-14.2(±2.2)	21.2(±5.7)	32.2(±7.2)	80.0(±1.5)
(C) Casein 10%	43.3(±4.9)	-	+18.0(±2.8)	57.0(±5.9)	94.2(±0.6)	97.4(±0.04)
(D) Non-protein	44.5(±0.5)	-	-10.0(±1.0)	34.4(±2.1)	-	-

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Table 4
Faecal Nitrogen and DNA of rats fed
on different diets (1st experiment)

Groups of rats	mg DNA/g faeces	mg N/g faeces	mg DNA/mg faecal N	mg DNA/mg dietary N
A	10.6 (±1.0)	48.6 (±2.2)	0.21 (±0.01)	0.20 (±0.05)
B	9.8 (±0.9)	50.4 (±2.3)	0.19 (±0.02)	0.19 (±0.06)
C	4.5 (±0.3)	24.2 (±2.2)	0.19 (±0.02)	0.02 (±0,00)
D	6.7 (±0.3)	20.4 (±0.8)	0.33 (±0.02)	-

(A) autoclaved and alcohol extracted beans

(B) autoclaved beans

(C) casein diet

(D) non-protein diet

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Table 5

Faecal nitrogen and DNA of rats fed on different diets(2nd experiment)

Groups of rats and diets	mg DNA/g faeces	mg N/g faeces	mg DNA/mg faecal N	mg DNA/mg dietary N
A	11.3 (±0.5)	50.7 (±4.7)	0.22 (±0.02)	0.17 (±0.02)
B	11.4 (±0.6)	54.8 (±3.6)	0.21 (±0.01)	0.15 (±0.01)
C	5.8 (±0.5)	25.8 (±1.4)	0.23 (±0.01)	0.02 (±0.004)
D	7.6 (±0.4)	20.0 (±0.0)	0.38 (±0.02)	-

(A) autoclaved and alcohol extracted beans

(B) autoclaved beans

(C) casein diet

(D) non-protein diet

(Table 6)
Weight of organs of rats fed beans and control diets
(1st experiment)

Rats & Diets	Weight of Pancreas		Weight of spleen		Weight of liver		Weight of kidneys		Weight of heart		Weight of adrenal	
	mg	mg/100g body Wt.	mg	mg/100g body Wt.	(g)	g/100g body Wt.	(g)	g/100g body Wt.	(g)	g/100g body Wt.	mg	mg/100 body Wt.
A	96.4 (±10.5)	457.4 (±31.6)	28.2 (±2.4)	135.8 (±9.7)	0.77 (±0.1)	3.66 (±0.19)	0.24 (±0.03)	1.12 (±0.06)	0.125 (±0.01)	0.59 (±0.03)	9.6 (±1.0)	45.8 (±4.4)
B	97.6 (±8.5)	460.0 (±37.3)	28.6 (±3.1)	135.6 (±12.4)	0.84 (±0.06)	4.01 (±0.17)	0.25 (±0.02)	1.19 (±0.01)	0.129 (±0.01)	0.61 (±0.04)	11.0 (±1.4)	49.2 (±2.7)
C	155.2 (±14.3)	327.8 (±18.6)	138.0 (±9.9)	294.6 (±8.8)	1.95 (±0.1)	4.17 (±0.22)	0.42 (±0.04)	0.90 (±0.01)	0.271 (±0.02)	0.58 (±0.02)	13.0 (±1.4)	27.8 (±3.6)
D	64.0 (±9.2)	275.2 (±12.6)	67.8 (±7.6)	293.6 (±21.4)	0.91 (±0.06)	3.98 (±0.33)	0.27 (±0.04)	1.17 (±0.06)	0.167 (±0.02)	0.74 (±0.11)	10.8 (±1.4)	47.0 (±6.7)

A. autoclaved and lacohol extracted beans,

B. autoclaved beans,

C. casein diet and,

D. non-protein diet

(Table 7)
Weights of organs of rats fed beans and control diet
(2nd experiment)

Rats & Diets	Weight of pancreas		Weight of spleen		Weight of liver		Weight of kidneys		Weight of heart		Weight of adrenal	
	mg	mg/100g body.Wt.	mg	mg/100g body Wt.	(g)	g/100g body Wt.	(g)	g/100g body Wt.	(g)	g/100g body Wt.	mg	mg/100g body Wt.
A	155.2 (±20.7)	448 (±62)	42.2 (±5.5)	121.7 (±21.7)	1.03 (±0.06)	2.97 (±0.27)	0.39 (±0.02)	1.12 (±0.1)	0.16 (±0.01)	0.46 (±0.07)	11.0 (±0.7)	31.5 (±4.5)
B	155.0 (±21.5)	475.5 (±64)	42.0 (±8.0)	131.0 (±29.1)	0.999 (±0.07)	3.07 (±0.16)	0.39 (±0.01)	1.21 (±0.03)	0.15 (±0.01)	0.45 (±0.04)	12.7 (±1.6)	39.2 (±5.2)
C	201.6 (±22.5)	330 (±29)	174.6 (±8.4)	287.0 (±21.2)	2.73 (±0.39)	4.44 (±0.19)	0.55 (±0.07)	0.90 (±0.02)	0.30 (±0.02)	0.49 (±0.03)	15.6 (±0.1)	25.0 (±4.3)
D	97.5 (±4.5)	282 (±9)	103 (±21)	299.5 (±45.5)	1.55 (±0.06)	4.5 (±0.25)	0.42 (±0.006)	1.21 (±0.03)	0.22 (±0.01)	0.63 (±0.02)	11.5 (±2.5)	33.5 (±6.5)

A. autoclaved and alcohol extracted beans,

B. autoclaved beans,

C. casein diet and,

D. non-protein diet

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