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MORE EVIDENCE ON THE PRESENCE OF AN UNKNOWN TOXIC SUBSTANCE(S) IN THE SAGABEAN

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ABSTRACT

Saga (Adenanthera pavonina Linn) tergolong kacang-kacangan (Leguminosae), maka diduga seperti juga hampir semua kacang-kacangan, mengandung faktor-faktor "anti-nutrisi", seperti trypsin inhibitor, fitohaemagglutinin dan saponin. Telah diketahui bahwa ada beberapa kacang-kacangan, yang di samping faktor "anti-nutrisi", juga mengandung zat beracun seperti: koro wedus (Dolichos lablab) dan kratok (Phaseolus lunatus), yang mengandung sianida (HCN), lamtoro (Leucaena glauca) yang mengandung mimosine, dan saga (Abrus precatorius Linn) yang mengandung racun abrin.

Timbul pertanyaan apakah biji saga juga mengandung racun, di samping faktor anti-nutrisi tersebut di atas.

Pada biji saga yang telah dikuliti, direndam, dicuci dan direbus dapat dianggap bahwa faktor-faktor anti-nutrisinya telah hilang. Tetapi walaupun demikian terbukti dengan percobaan tikus putih muda, bahwa biji saga yang telah diolah tetap hanya dimakan sedikit saja, juga bila ditambah dengan methionine dan threonine, dua asam amino yang sudah diketahui sangat terbatas kadarnya dalam protein biji saga.

Dari percobaan ini dapat ditarik kesimpulan, bahwa dalam biji saga juga terdapat suatu zat toksik (racun) yang tidak dapat dihilangkan dengan cara pengolahan tersebut di atas.

INTRODUCTION

In our previous publication we have mentioned the existence of an unknown toxic or anti-nutritive factor in a legume seed, the saga bean *(Adenanthera pavonina* Linn). Saga meal was obtained from saga bean boiled in the skin. This meal was fed to young albino rats as a single source of protein (at 10% level), but it was almost completely rejected by the young rats resulting in starvation. Three possibilities of this refusal were considered; these were either anti-nutritive factors, which may still remain in the saga meal, an inadequate amino-acid pattern of the saga meal or thirdly a toxic factor which does not belong to the 2 groups mentioned before.

New feeding experiments were therefore carried out but now with saga meal, processed in such a way, that the anti-nutritive factors can be considered to be fully inactivated or eliminated. The possibility of an inadequate amino-acid pattern was overcome by the supplementation with DL–methionine and L–threonine.

MATERIAL

Whole, raw saga beans were crushed in a coffee mill for easier decortication. The husked split beans (cotyledons) were steeped in water for 36 hours and the steeping water was frequently changed. During each steeping foaming of the steeping water was continually observed, especially after 12 hours of soaking when the

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cotyledons were completely swollen.

After 36 hours, the soaked split decorticated beans were boiled in sufficient water for at least one hour. After boiling the beans were drained and ground in a meat mill and dried in an oven overnight at 60° – 70°C. This coarse powder was further ground in a coffee mill to a fine powder for better incorporation into experimental ratdiets.

METHODS

a) Chemical analysis:

- The proximate principles were determined according to the methods of AOAC (1975).
- The amino-acids analysis was carried out by the Biochemistry Division of the Nutrition Institute of the Mahidol University, Bangkok (Thailand).

b) Rat feeding Experiments:

- All the rat feeding experiments were carried out with young weanling albino rats of the Lembaga Makanan Rakyat (L.M.R.) strain Wistar derived, bred in our Nutrition Research Unit Diponegoro.
  The composition of the experimental diets prepared is given in the Addendum Table 1.
  The PER–study was carried out according to AOAC.
- Supplementation with DL–methionine and L–threonine (both from E. Merck, Darmstadt) was done on the soy and on the saga experimental diet. The quantity added was the level as mentioned in the reference provisional amino-acid pattern of FAO/WHO 1972/73.

RESULTS

1. Chemical analysis:

a) Proximate principles

A comparison of the proximate principles of the saga meals treated in two different ways, can be seen from table 1. (See Table 1).

(process A : boiling the whole saga beans while in the seed coat.
process B: first decortication, followed by steeping and repeated changes of fresh steeping water and then at the end boiling).

The average net yield of saga meal obtained by process B is much lower, i.e. about 219 g as compared to about 340 g by process A, per kg of raw saga beans. A loss of about 130 g (equal to 40%) of total solids must have taken place during the process of steeping and repeated washings. What are lost in the foaming steeping water are most probably soluble carbohydrates, proteins and minerals. Fat was not lost during the treatment, because the fat content of the saga meal B is 36% which is twice the fat content of saga meal A with 18% only. The increase in fat content in saga meal B agrees well with the estimated loss of total solids of 40%.

Table 1. Proximate Principles of 2 kinds of sagameal and soy (in grams per 100 gram)

(Average per 100 g)		Saga meal process A	Saga meal process B	Soy meal
Moisture	%	4.8	4.2	5.3
Nitrogen	%	6.06	4.68	6.50
Protein (Nx5.75)	%	34.6	26.7	37.1
Fat	%	18.0	36.1	20.0
Carbohydrate (by difference)	%	38.2	30.1	33.7
Fibre	%	(12.6)	–	4.0
Minerals	%	4.4	2.9	3.9
Avg. net yield : from 1 kg of raw saga bean (as meal)	g	±340	±210	
	%	± 34	± 21	

Description of process :

Process A : Boiling of whole saga beans and afterwards decortication. (our previous report, 1981).

Process B : Crushing and decortication of saga beans; soaking under repeated washing of cotyledons and afterwards boiling. (this report)

b) Amino-acid pattern:

From the comparison of the essential amino-acid content as given in table 2, it is clear that the amino-acid pattern also is unfavorably influenced by the process as reported under B.

2. Rat feeding experiments:

The results of the rat feeding experiments carried out as a Protein Efficiency Ratio Study (growth study), is summarized in Table 3.

The growth of the rats and their cumulative intake of food throughout the 4-weeks study, were presented as graphs no. 1 and no. 2. in the Addendum.

Table 2. Comparison of the essential amino-acid contents of 2 kinds of saga meal and soy bean. (in milligrams per gram of Nitrogen)

Essential amino-acids	Provisional amino-acid pattern FAO/WHO 1973 1)	Saga meal process A 2)	Saga meal process B 3)	Soy 1)
1. Isoleucine	250	350	149	290
2. Leucine	440	600	437	494
3. Lysine	340	460	359	391
4. Methionine	.	60	55	84
5. Cystine	.	100	53	81
Total s–c	220	160	108	165
6. Phenylalanine	.	437	251	341
7. Tyrosine	.	342	202	165
Total arom	380	779	453	506
8. Threonine	250	230	127	247
9. Tryptophan	65	–	29	76
10. Valine	310	385	161	291

Reff. :
1) Food Composition Table for use in East Asia FAO/US.–HEW.p.193. (1972)
2) Oey. et. al 1981. Amino-acid determination by Nat. Inst. for Chemistry, Indon. Inst. of Sciences (LIPI). – Bandung.
3) Amino-acid determination by the Inst. of Nutrition, Mahidol University, Bangkok, Thailand. (1983).

Table 3. Summary of Results of the Ratfeeding Experiments with Saga meal (PER–study)

XP–diets*	Number of XP–rats	Avg. Starting weight	Protein Content of XP-diet	Total Weight gain 4 weeks	Avg. Total Food Intake per rat	Avg. Total Protein per rat	PER ± SD
	n	g	%	g	g	g	
Protein free	6	47.9	–	–16.8	59.3	0	–
Skin milk	6	47.9	9.81	+ 49.9	174.6	17.13	2.91 ±0.26
Soy, boiled	6	47.9	9.52	+ 45.5	194.0	18.47	2.46 ±0.24
Soy + Me, Th	10	48.0	9.23	+ 63.1	203.3	18.76	3.35 ±0.32
Saga, Dc SB	6	47.9	10.29	–11.9	67.0	6.89	(–1.87±0.69)
Saga, Dc SB + Me, Th.	10	48.0	10.22	+ 2.2	84.8	8.67	(+ 0.24±0.34)

Dc SB = Decorticated Steeped Boiled
Me Th = Methione + Threonine

*) The experimental diets (except the protein free diet) contain approx. 10% protein with either skimmilk, soy or saga protein as the single (only) source of protein
(For more details please see Addendum Table 2)

As can be seen from the summary on Table 3, the rats on the protein-free diet and on the 2 experimental saga diets with 10% protein each, did not gain in weight; on the contrary they lost weight. The PER therefore cannot be calculated, as the value will be negative.

The rats on the diets based on skim milk, soy and soy supplemented with methionine and threonine were eaten well by the rats and they gained significantly in weight. This is best illustrated by the growth curves as presented in graph 1. Although the 2 saga-diets do contain 10% of protein, these two diets were consumed in a low quantity only, comparable to that of the protein-free diet. Supplementation with methionine and threonine did not improve the food intake and the growth of the experimental rats. One might reasonably expect that the supplementation to the treated saga meal would give results which will be of the same order as soy.

The growth curves and the curves on the cumulative intake of food is given in the Addendum Graphs 1 and 2.

DISCUSSION

The process of decortication and steeping for 36 hours under frequent changes of water followed by boiling must be regarded as sufficient to eliminate the anti-nutritive factors present in the raw saga bean, of which the trypsin inhibitor, the phyto-agglutinins and the saponins are the best known.

An extensive authoritative review is given by various authors in "Toxic Constituents of Plant Foodstuffs" Edited by Irvin E. Liener (1969) Academic Press, from which valuable information has been obtained. These authors pointed out, that trypsin inhibitor and phyto-agglutinins are heat-labile and are destroyed by adequate cooking of the legumes. In our experiment, boiling in water of the saga beans for one hour is regarded as sufficient to eliminate these two factors.

The saponins in the saga meals treated according to process B is very much reduced in quantity as seen from the excessive foaming and the loss in total solids. Muchtadi (1982) has studied extensively the chemistry of saga saponins. According to him, the saga saponins are probably of *triterpenic nature* just like that of soybean.. The quantity expresses in dry material is close to that of soybean viz. 0.65 – 1.18 g%.

Saponins are hemolytic and toxic to warm-blooded animals when injected into the blood stream. According to Birk (1969) ingested soybean saponins are not absorbed into the blood stream and they are decomposed by the caecal microflora. The same may also happen with saga saponins. We may therefore conclude that the role of anti-nutritive factors can be excluded when considering the rejection of the saga experimental diets by the experimental rats. The supplementation with amino-acids did not improve the very poor food intake. One is therefore inclined to conclude that the rejection of the experimental saga diet prepared as mentioned under process B, must be due to the presence of an as yet unknown toxin or toxic substance (s), still present in the saga meal.

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Addendum

Tabel 1. Composition of various Diets for Ratfeeding Experiment

PER - Study

XP diet Protein content as planned:	Protein free 0%	Skim milk 10%	Soy 10%	Soy +Me+Thr 10%	Saga boiled 10%	Saga boiled +Me+Thr 10%
Nitrogen Content %	0	1.54	1.67	1.62	1.80	1.79
Protein Content %	0	9.81	9.52	9.23	10.29	10.22
Composition in g per kg XP–diet						
1. Fat to be added	100	98	36	36	–	–
2. Starch	720	526	608	608	555	555
3. Glucose	150	50	50	50	50	50
4. Salt mixture	20	20	20	20	20	20
5. Vitamin mixture	+	+	+	+	+	+
6. DL–Methionine	–	–	–	0.962	–	1.954
7. L–Threonine	–	–	–	0.052	–	2.145
8. Celluflour	10	20	–	–	–	–
9. Skim milk	–	286	–	–	–	–
10. Soy, boiled, dried	–	–	286	286	–	–
11. Saga, soaked, boiled	–	–	–	–	375	375
Total	1000	1000	1000	1000	1000	1000

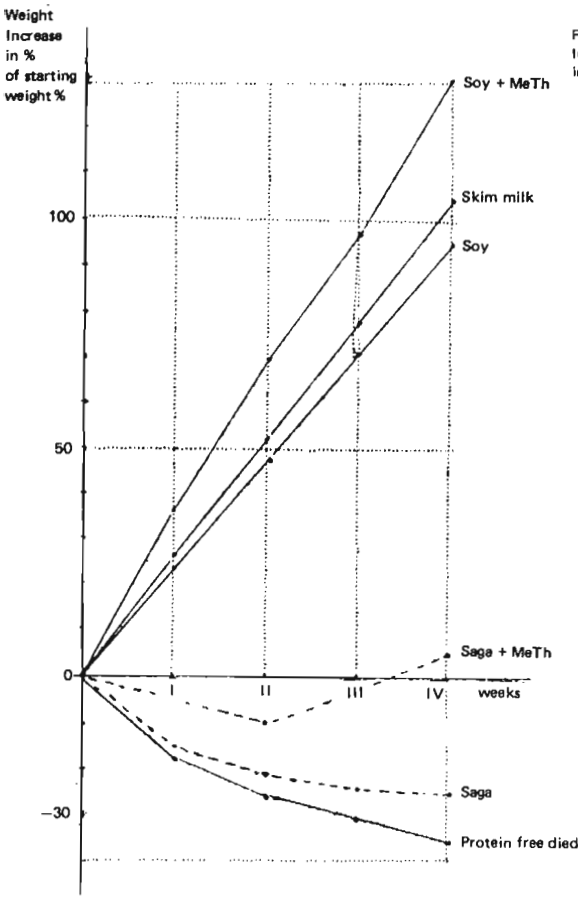
Table 2. Summary of Results of Protein Efficiency Ratio Determination per rat by weeks of: Saga beans, decorticated, soaked and boiled as a single sourge protein.

Experimental Diet	no. of rats	Protein content of XP–diet (N x 5.71)	Avg. Wght at Start	Avg. Cumulative Wght. Increase in % of Starting Weight at End of week no.				Total Wght. Gain	Avg. Total Food–Intake per rat by weeks during week no.				Avg. Total Food Intake	Avg. Total Protein Intake	PER ±SD
	n	g%	g	I %	II %	III %	IV %	g	I g	II g	III g	IV g	g	g	
Protein-free	6	–	47.9	–17.2	–25.4	–30.5	–35.2	–16.8	18.3	14.8	13.4	12.8	59.3	0	–
Skim milk (Nx6.38)	6	9.81	47.9	26.7	52.2	77.7	104.5	49.9	36.8	44.3	47.8	45.7	174.6	17.13	2.91 ±0.26
Soy, boiled	6	9.52	47.9	23.7	47.7	71.3	94.9	45.5	41.7	48.9	54.4	48.9	194.0	18.47	2.46 ±0.24
Soy, boiled + Me + Th	10	9.23	48.0	36.5	69.5	96.9	130.8	63.1	45.6	53.0	53.7	51.1	203.3	18.76	3.35 ±0.32
Saga, Dc, S.B.	6	10.29	47.9	–14.8	–20.8	–23.7	–25.0	–11.9	15.6	16.5	18.1	16.8	67.0	6.89	(–1.87±0.69)
Saga, Dc, S.B. + Me + Th	10	10.22	48.0	– 3.8	–10.2	– 3.2	+ 4.8	+ 2.2	20.0	17.0	23.8	24.0	84.8	8.67	(+0.24 ±0.34)

Saga DcSB = Saga Decorticated Soaked (Steeped) Boiled.
Me + Th = Methionine + Threonine

Addendum

Graph no. 1. Growth curves of young weanling albinorats fed various experimental diets (10% protein).



Addendum

Graph no. 2. Cumulative Intake of Food of young weanling albino-rats on various experimental diets (at 10% protein level)