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NUTRIENT AND TRYPSIN INHIBITOR CONTENT OF HYDROPONICALLY SPROUTED SOYA BEANS

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ABSTRACT

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Soya bean seeds were sprouted hydroponically in the light at 21°C for 1—7 days. Samples were freeze-dried, ground through a 1-mm screen and analyzed for proximate nutrients, amino acids, minerals and trypsin-inhibitor content. During sprouting, quantities of energy, dry matter (DM), total lipids (TL) and starch (NFE) decreased significantly ($P < 0.05$). Protein weight did not change during sprouting. Weight of ash increased slightly and weight of fibre increased markedly during sprouting. Among the amino acids, weight of glutamic acid decreased whilst weights of aspartic acid and leucine increased. There was a slight gain in weights of Cu, Na and Zn, these being related to the mineral content of the water source. Weight of trypsin inhibitor (TI) decreased cubically with increased sprouting time.

Although nutrients are lost to a lesser degree during the sprouting of soya beans as compared with barley, the substantial reduction in TL may mean that the energy value is more adversely affected. It is not known whether the decrease in TI is great enough to render the sprouts safe for direct consumption by farm animals.

INTRODUCTION

Peer and Leeson (1985a,b) have recently described the nutrient composition and feeding value of barley grown for 1—7 days under hydroponic conditions. Although there was a significant loss in most major nutrients with sprouting, the process may have application to soya beans as an on-farm method of processing.

Owing to the recent interest in soya bean sprouts for human consumption, several researchers have investigated their nutrient concentration (Fordham et al. 1975; Kylen and McCready, 1975; Bates et al., 1977). In most of these studies soya beans have been sprouted for only 3—5 days in the dark and no work has been done with beans germinated hydroponically in the light in modern growth chambers.

Raw soya beans contain protease inhibitors which affect animal perfor-

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mance (Bowman, 1944; Ham and Sandstedt, 1944; Carver et al., 1946; Gertler et al., 1967; Hull et al., 1968; Latshaw et al., 1974; McNaughton et al., 1980, 1981). If germination of soya beans reduces the amount of inhibitor, raw sprouts could be given directly to farm animals. Studies on trypsin-inhibitor concentration of germinating soya beans have proved inconclusive. Freed and Ryan (1978) and Tanimura et al. (1980) reported a decrease in trypsin inhibitor activity, while Collins and Sanders (1976) and Bates et al. (1977) found little or no difference between sprouted and non-sprouted soya beans. It was decided to determine the nutrient composition and the trypsin inhibitor activity of soya beans sprouted hydroponically in the light for 1—7 days. Although it is known that vitamin concentration of grains change during germination (Wai et al., 1974; Vanderstoep, 1981) these nutrients were not assayed in this study.

MATERIALS AND METHODS

Hydroponic unit

Soya bean seeds were germinated in a hydroponic sprouting chamber using the equipment previously described by Peer and Leeson (1985a). The seed was sprayed with water for 15 min every 4 h and the temperature maintained at 21°C. A nutrient solution was not added to the spray water.

Seed preparation

Soya beans (800 g) were pre-wetted with an equal volume of water, then distributed on 91.9 × 30.5-cm white plastic trays within the hydroponic unit. Growth period was from 1 to 7 days. Samples were collected each day, freeze-dried, ground through a 1-mm mesh screen and stored in tightly sealed glass jars at room temperature. Three samples of dry soya beans were prepared similarly to serve as controls. All nutrient and trypsin-inhibitor analyses were conducted on the dry, ground samples.

Nutrient analysis

Dry matter (DM), crude protein (CP), crude fibre (CF) and mineral contents of sprouted and non-sprouted soya beans were determined according to Association of Official Analytical Chemists (1975). Nitrogen-free extract (NFE) was calculated by subtracting the summation of CP, CF, fat and ash from 100. Cystine and methionine were determined by the performic acid procedure reported by Hirs (1967). The remaining amino acids were analyzed according to the instruction manual for the Beckman Amino Acid Analyzer, Model 119B (1975).

Dry-matter loss (DML) was determined by sprouting three trays of 300 g soya bean seed and drying the entire contents of the trays. Three, 300-g samples of dry seed were freeze-dried and their average dry-matter weight served as a control. The DML was assumed to be the difference between the DM weight of the control and test samples.

Trypsin-inhibitor analysis

Trypsin-inhibitor (TI) analysis was conducted on dry samples according to the procedure developed by Hamerstrand et al. (1981). This method involved reaction of sample alkali homogenates with trypsin and benzolyl-DL-arginine-p-nitroanilide in Tris (hydroxymethyl) aminomethane buffer. The reaction was terminated after 10 min by the addition of 30% acetic acid. Absorbance of solutions was determined at 410 nm (Pye Unicam Sp6-5050).

Statistical analysis

A completely randomized design with three replications of eight treatments of growing period was employed. The relationship between growth period of 1—7 days and nutrient and trypsin-inhibitor concentrations and weights was analyzed using orthogonal polynomials (Steel and Torrie, 1980). Regression equations were also determined. All data from the analysis are expressed on a dry-matter basis, unless otherwise stipulated. Day 0 equals unsprouted soya beans in all tables.

RESULTS AND DISCUSSION

Germination of the soya beans was about 96%. The white tip of the protruding radicle is visible after 24 h in the hydroponic unit. It is difficult to distinguish between radicle and hypocotyl until after 3 days of sprouting, by which time the hypocotyl has turned green. After 5 days, the seed coat has broken off and the cotyledons have separated slightly. The cotyledons are open and the first primary leaf is barely visible after 7 days.

Proximate analysis

By Day 7, there was an increase in concentrations of ash, crude fibre (CF) and crude protein (CP) by 18.6%, 44.1%, and 7.8%, respectively, relative to unsprouted beans. Nitrogen-free extract (NFE) and total lipids (TL) decreased by 4.2% and 20.4%, respectively, by 7 days of sprouting. Similar changes in nutrient profile were observed by Fordham et al. (1975) and Kylen and McCready (1975).

The actual change in nutrient weights during sprouting is not clear until dry-matter loss has been considered. By Day 7, only 93.1% of the DM, 78.4% of the NFE and 74.1% TL remained (Table I).

Oil is a major energy source for the developing soya bean embryo during

TABLE I

Nutrient weights of 300 g of soya beans sprouted for various lengths of time

Nutrient	Day of sprouting 0	1	2	3	4	5	6	7	Regression coefficient ± S.E.	Regression constant	RSD	R^2 / $P <$
DM (g)	280	277	279	271	271	264	260	261	$-3.15x$ ± 0.219	281	2.46	0.90
S.E.	±0.1	±0.9	±0.3	±0.5	±0.5	±0.2	±0.7	±2.5				0.0001
Ash (g)	15.5	15.3	15.8	16.1	16.2	16.6	17.0	17.7	$+0.32x$ ± 0.042	15.2	0.48	0.72
S.E.	±0.12	±0.17	±0.40	±0.23	±0.43	±0.37	±0.29	±0.21				0.0001
CF (g)	14.6	14.2	14.4	14.5	16.2	16.9	18.8	26.6	$-1.30x$ ± 0.436	15.0	1.35	0.87
S.E.	±0.14	±0.15	±0.79	±0.24	±0.19	±0.14	±1.37	±1.21	$+0.36x^2$ ± 0.060			0.0001
CP (g)	112	109	78.0	108	109	112	111	113	$+1.41x$ ± 1.84	102	20.7	0.03
S.E.	±0.5	±0.5	±33.6	±0.4	±1.2	±3.3	±0.9	±1.5				0.453
G.E. (MJ)	5.48	5.47	5.47	5.23	5.18	4.97	4.72	4.62	$-0.03x$ ± 0.025	5.51	0.076	0.95
S.E.	±0.038	±0.009	±0.032	±0.036	±0.025	±0.006	±0.016	±0.073	$-0.02x^2$ ± 0.003			0.0001
NFE (g)	76.2	74.8	74.3	74.0	71.5	65.4	67.9	59.8	$-2.13x$ ± 0.344	77.9	3.86	0.64
S.E.	±1.58	±0.70	±1.63	±1.36	±1.55	±3.41	±3.05	±2.36				0.0001
TL (g)	62.1	63.4	62.7	57.8	57.2	53.1	45.6	46.0	$-0.39x$ ± 0.786	63.2	2.42	0.89
S.E.	±1.32	±0.59	±1.22	±1.32	±1.12	±0.78	±0.89	±1.45	$-0.34x^2$ ± 0.108			0.0001

germination. The catabolism of oil produces glycerol and fatty acids, which are used to provide carbohydrates for respiration and cell wall synthesis (Hsu et al., 1973). Storage carbohydrates are used to a lesser degree. The 21.6% and 25.9% reduction in weights of NFE and TL by Day 7 reflects the extent to which these energy reserves are utilized by the developing seedling.

The amount of dry matter lost during sprouting will depend on the energy source utilized. In barley, the major component by weight is starch (Peer and Leeson, 1985a). The 34% decrease in amount of starch, therefore, accounted for the large decrease in dry matter after 7 days of sprouting. In soya beans, the major energy reserve is oil, which is a more concentrated source of energy (Hamilton and Vanderstoep, 1979). The 6.9% dry-matter loss in soya beans, as compared to 18% loss in barley, after 7 days of sprouting reflects this situation.

Unlike barley, soya beans sprouted for 7 days did not change significantly in weight of protein (Table I). Hurst and Sudia (1973) reported no change in nitrogen weight of soya beans germinated in the dark, while soya beans given a nutrient solution and germinated in the light were able to increase their nitrogen weight by assimilating the nitrogen from the culture medium.

Fibre weight increased with seedling growth owing to the increase in cell size and numbers, (James, 1940; Mayer and Poljakoff-Mayber, 1975).

Amino acids

Table II indicates the weights of amino acids present after sprouting 300 g of soya beans. There was a significant linear decrease in weights of alanine, arginine, threonine, glycine, lysine, proline and serine, while weights of aspartic acid and leucine increased linearly. Weights of histidine, isoleucine, methionine, phenylalanine and tyrosine did not change during sprouting (Table II).

Since the total amount of protein did not change during sprouting, the change in amino acid weights was merely a shift in amino acid profile. Glutamic acid is used as a major source of nitrogen for amino acid synthesis (Folkes and Yemm, 1958). Aspartic acid is formed from the decarboxylation of glutamic acid (Bidwell, 1974), which explains the increase in aspartic acid seen here.

Minerals

Table III illustrates the changes in mineral weights which occur when 300 g of soya beans are sprouted for up to 7 days. Weight of Cu increased linearly with increased sprouting time, while weights of Na and Zn increased cubically. The increase in weights of these minerals could be due to the amount of Na in the water supply used (Peer and Leeson, 1985a) and leaching of Cu and Zn from the metallic racks and nozzles of the hydro-

TABLE II

Amino acid weights (g) after sprouting 300 g of soya beans for various lengths of time

Amino acid	Day of sprouting							
	0	1	2	3	4	5	6	7
Alanine	4.66	4.66	4.79	4.37	4.03	3.25	3.04	3.12
S.E.	±0.340	±1.593	±0.072	±0.150	±0.270	±0.285	±0.459	±0.283
Arginine	7.21	7.09	7.20	6.54	5.93	5.06	4.50	4.44
S.E.	±0.494	±2.460	±0.161	±0.234	±0.256	±0.218	±0.655	±0.339
Aspartic acid	12.3	12.2	9.05	13.2	14.2	14.0	17.5	21.9
S.E.	±0.78	±4.21	±3.96	±0.286	±1.11	±1.07	±2.67	±2.11
Cystine	0.77	1.09	0.94	0.88	0.83	0.73	0.80	0.81
S.E.	±0.094	±0.116	±0.008	±0.007	±0.019	±0.036	±0.069	±0.261
Glutamic acid	19.1	19.4	19.6	17.3	14.8	10.4	8.57	7.92
S.E.	±1.20	±6.59	±0.211	±0.58	±1.01	±1.10	±1.39	±0.876
Glycine	4.69	4.67	4.75	4.37	4.02	3.11	2.83	2.91
S.E.	±0.327	±1.598	±0.036	±0.145	±0.262	±0.308	±0.383	±0.238
Histidine	2.91	3.07	3.03	2.89	2.87	2.35	2.49	2.66
S.E.	±0.178	±0.823	±0.021	±0.075	±0.194	±0.134	±0.319	±0.090
Isoleucine	4.45	5.20	4.97	4.67	4.48	4.19	4.36	4.57
S.E.	±0.152	±1.765	±0.063	±0.144	±0.276	±0.026	±0.483	±0.178
Leucine	4.45	5.20	4.97	4.67	7.97	7.02	6.85	7.08
S.E.	±0.152	±1.765	±0.063	±0.144	±0.432	±0.340	±0.821	±0.409
Lysine	7.06	6.96	7.08	6.50	5.95	4.48	4.14	4.29
S.E.	±0.504	±2.337	±0.094	±0.177	±0.381	±0.419	±0.577	±0.265
Methionine	1.54	1.85	1.59	1.51	1.42	1.29	1.35	1.48
S.E.	±0.164	±0.206	±0.027	±0.021	±0.039	±0.073	±0.078	±0.443
Phenylalanine	5.51	5.36	5.50	5.14	4.94	4.34	4.31	4.37
S.E.	±0.444	±1.800	±0.064	±0.146	±0.317	±0.219	±0.481	±0.219
Proline	5.54	5.56	5.61	5.72	4.85	3.80	3.24	3.23
S.E.	±0.376	±1.843	±0.090	±0.262	±0.341	±0.348	±0.453	±0.219
Serine	4.98	5.10	5.30	4.95	4.51	3.61	3.46	3.51
S.E.	±0.290	±1.687	±0.061	±0.157	±0.254	±0.309	±0.490	±0.285
Threonine	4.31	4.23	4.33	3.96	3.52	2.97	2.90	3.00
S.E.	±0.303	±1.484	±0.110	±0.097	±0.173	±0.288	±0.416	±0.170
Tyrosine	3.65	4.02	4.12	3.89	3.97	3.46	3.10	3.07
S.E.	±0.201	±1.350	±0.059	±0.135	±0.300	±0.208	±0.423	±0.192
Valine	6.17	5.82	6.90	6.06	5.90	4.75	4.54	5.11
S.E.	±0.123	±1.684	±0.454	±0.206	±0.442	±0.407	±0.609	±0.204

ponic chamber. Weight of Fe decreased linearly, while weight of P decreased cubically over time. Calcium and Mg weights did not change during sprouting (Table III). Since no nutrient solution was used, the increase in mineral weights must be due to the amount of minerals in the water supply.

Table IV summarizes changes in the weights of major nutrients when 1 kg soya bean dry matter is germinated.

Regression coefficient ± S.E.		Regression constant	RSD	R^2 $P <$
-0.28x	± 0.086	4.98	0.960	0.33 0.003
-0.47x	± 0.127	7.63	1.420	0.38 0.001
+1.30x	± 0.375	9.74	4.209	0.35 0.002
-0.02x	± 0.017	0.93	0.191	0.07 0.219
-1.93x	± 0.357	21.4	4.00	0.57 0.0001
-0.32x	± 0.085	5.04	0.956	0.39 0.001
-0.08x	± 0.046	3.06	0.517	0.12 0.099
-0.07x	± 0.091	4.85	1.016	0.03 0.450
+0.43x	± 0.121	4.52	1.36	0.37 0.002
-0.50x	± 0.125	7.55	1.401	0.42 0.0006
-0.05x	± 0.027	1.67	0.302	0.12 0.100
-0.20x	± 0.093	5.64	1.045	0.18 0.042
-0.41x	± 0.103	6.11	1.153	0.42 0.0007
-0.29x	± 0.091	5.42	1.023	0.31 0.005
-0.24x	± 0.078	4.50	0.873	0.30 0.005
-0.13x	± 0.073	4.10	0.824	0.12 0.101
-0.24x	± 0.102	6.51	1.150	0.20 0.026

Trypsin inhibitor

There was a highly significant ($P < 0.002$) cubic relationship between growth period and TI weight (Table V). The regression equation is $y = 58.6 - 4.46x + 2.12x^2 - 0.23x^3$, where y = TI (mg g^{-1} dry matter) and x = time (days). Day 7 sprouts contained 12.7% less TI by weight than the unsprouted beans.

Several researchers have investigated the effect of germination on TI

TABLE III

Mineral weights after sprouting 300 g soya beans for various lengths of time

Mineral	Day of sprouting								Regression coefficient ± S.E.		Regression constant	RSD	R^2 / $P <$
	0	1	2	3	4	5	6	7					
Ca (g)	0.72	0.73	0.69	0.70	0.69	0.65	0.69	0.74	$-0.002x$	± 0.0052	0.71	0.0589	0.005
S.E.	±0.066	±0.052	±0.025	±0.010	±0.008	±0.023	±0.009	±0.040					0.740
Cu (mg)	2.33	3.01	3.95	4.76	5.38	5.27	6.61	7.60	$+0.71x$	± 0.043	2.38	0.485	0.92
S.E.	±0.214	±0.071	±0.149	±0.251	±0.289	±0.172	±0.075	±0.546					0.0001
Fe (mg)	3.37	3.30	3.37	3.07	3.47	2.53	2.50	2.87	$-0.11x$	± 0.035	3.46	0.389	0.33
S.E.	±0.145	±0.173	±0.426	±0.186	±0.167	±0.067	±0.100	±0.033					0.003
K (g)	6.05	5.24	5.73	5.37	5.18	4.81	5.16	5.56	$-0.38x$	± 0.123	5.98	0.379	0.37
S.E.	±0.031	±0.393	±0.102	±0.081	±0.190	±0.174	±0.180	±0.102	$+0.04x^2$	± 0.017			0.007
Mg (g)	0.72	0.75	0.73	0.74	0.72	0.71	0.75	0.76	$+0.002x$	± 0.002	0.73	0.026	0.03
S.E.	±0.009	±0.016	±0.010	±0.010	±0.019	±0.015	±0.009	±0.023					0.419
Mn (mg)	7.98	8.48	8.46	8.29	8.35	8.21	7.93	7.81	$-0.05x$	± 0.031	8.38	0.350	0.12
S.E.	±0.113	±0.164	±0.228	±0.187	±0.349	±0.100	±0.021	±0.067					0.095
Na (g)	0.21	0.42	0.40	0.51	0.55	0.55	0.73	0.86	$+0.18x$	± 0.040	0.23	0.055	0.93
S.E.	±0.038	±0.027	±0.013	±0.033	±0.010	±0.026	±0.031	±0.036	$-0.04x^2$	± 0.014			0.0001
									$+0.004x^3$	± 0.0013			
P (g)	2.10	2.11	2.12	2.08	2.07	1.92	2.01	2.08	$+0.09x$	± 0.040	2.09	0.055	0.52
S.E.	±0.028	±0.007	±0.018	±0.033	±0.039	±0.006	±0.043	±0.018	$-0.04x^2$	± 0.013			0.001
									$+0.004x^3$	± 0.0013			
Zn (mg)	18.0	20.2	22.3	24.3	26.6	24.6	29.6	34.1	$+4.14x$	± 1.444	17.6	1.98	0.87
S.E.	±0.27	±0.33	±0.091	±0.952	±1.71	±1.17	±0.31	±1.96	$-1.00x^2$	± 0.502			0.0001
									$+0.11x^3$	± 0.05			

TABLE IV

Nutrient weights (g) during the sprouting of 1 kg of soya bean dry matter

Day	DM		Ash		CF		CP		Lys		NFE		T.L.	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
0	1000	±0.0	55.2	±0.43	52.1	±0.52	398	± 1.9	25.1	±1.80	272	± 5.6	222	±4.7
1	989	±3.4	54.7	±0.59	50.7	±0.52	391	± 1.7	24.8	±8.33	267	± 2.5	226	±2.1
2	994	±1.1	56.4	±1.41	51.4	±2.82	397	± 2.9	25.3	±0.34	265	± 5.8	223	±4.4
3	967	±1.6	57.4	±0.83	51.5	±0.90	388	± 1.4	23.2	±0.63	264	± 4.8	206	±4.7
4	965	±1.7	57.9	±1.52	57.8	±0.67	391	± 4.2	21.2	±1.37	255	± 5.5	204	±4.0
5	942	±0.8	59.4	±1.30	60.2	±0.49	400	±11.8	14.3	±0.48	233	±12.2	190	±2.8
6	929	±2.6	60.8	±1.02	67.0	±4.91	397	± 3.4	14.8	±2.06	242	±10.6	163	±3.2
7	930	±8.7	63.1	±0.75	87.8	±4.32	401	± 6.1	15.3	±0.94	213	± 8.40	164	±5.13

DM = Dry matter; CF = crude fibre; CP = crude protein; Lys = lysine, NFE = nitrogen free extract; TL = total lipids; S.E. = standard error of the mean.

TABLE V

Trypsin inhibitor (TI) weights of soya beans sprouted for up to 7 days

Day	TI (mg g^{-1} dry matter)	
	Mean	S.E.
0	58.6	± 0.91
1	56.1	± 1.26
2	55.9	± 2.36
3	58.3	± 0.84
4	60.1	± 1.92
5	59.1	± 1.45
6	58.7	± 1.90
7	51.1	± 2.53

Day 0 = unsprouted soya beans.
Regression equation: $y = 58.6 - 4.47x + 2.12x^2 - 0.23x^3$, where y = TI (mg g^{-1} dry matter) and x = time (days); $P < 0.002$, $R^2 = 0.51$; residual standard deviation = ±2.777.

activity in soya beans. Often, there is a reduction in the activity of TI, with the decrease being dependent on the growth period and the method of analysis employed. Collins and Sanders (1976), after germinating soya beans for 3 days, reported a small decrease in the amount of TI activity, which was attributed, in part, to the leaching of TI during daily washings. Freed and Ryan (1978), using an immunoelectrophoretic TI assay, found a 13% and 50% reduction in the concentration of Kunitz TI on a dry and wet basis, respectively, after sprouting soya beans in the dark for 9 days. Bates et al. (1977) reported a 33% reduction in TI on a fresh basis, after 4 days of sprouting.

The decrease in the amount of TI on a dry-matter basis during the hydroponic sprouting of soya beans for 7 days seen here is comparable to the results of Freed and Ryan (1978). If this decrease during sprouting reduces the weight of TI to a safe level, sprouted soya beans could be given to livestock as a protein supplement directly on the farm, since protein is not lost during sprouting and the dry-matter loss is small. However, unlike the situation seen with barley (Peer and Leeson, 1985a), this loss of DM represents a substantial reduction in TL and hence energy values will be more adversely affected.

Limited research concerning the feeding value of sprouted soya beans has been conducted in the past. Everson et al. (1944) compared growth rate of rats fed on raw immature and mature soya beans sprouted for 60 h at 26°C. Rats fed on raw sprouted soya beans gained more weight than rats fed on raw mature soya beans, but less than those fed on raw immature beans. Autoclaving improved the digestibility of all three bean preparations. Mattingly and Bird (1945) gave 5-day sprouts to rats and chicks. The rat experiment confirmed the results of Everson et al. (1944), although the chick trial resulted in no difference in growth rate for chicks fed on sprouted or mature beans. Desikacher and De (1950), using rats, found that the biological value of 3-day sprouted soya beans was higher than that of raw soya beans, but observed no difference in TI levels of raw and sprouted beans. These feeding trials suggest that sprouting of soya beans improves their feeding value, even if weight of TI is not affected.

The TI analysis of the sprouted soya beans, reported herein, was conducted according to Hamerstrand et al. (1981), so limited results are available for direct comparison owing to the methodology of TI assay. Kakade et al. (1974) obtained a value of 12.0 trypsin units inhibited (TIU) per mg sample for commercial soy protein concentrate. Zamora and Veum (1979) obtained values of 1.2 TIU mg^{-1} and 7.1 TIU mg^{-1} of dry sample for heated unfermented and fermented soya beans, respectively. When the results from the analyses reported herein are converted to TIU mg^{-1} dry matter, a value of 93.5 TIU mg^{-1} is obtained for Day 7 sprouts, representing the lowest level of TI of all sprouts analyzed. Hamerstrand et al. (1981) obtained a value of 28.2 mg TI g^{-1} dry matter for a heat-treated sample of soya bean meal. The value for Day 7 sprouts, when expressed in the same units, is 51.1 mg TI g^{-1} dry matter.

From these comparisons, it would seem that the amount of TI in sprouts is still too high for their direct consumption by livestock. Feeding trials with different sprout ages and species of livestock need to be conducted to determine the digestibility of the sprouts and their effect on animal performance. In view of the results obtained with regular soya beans and the fact that sprouting has only a limited influence on amount of TI, it does not seem advisable or economical to use raw sprouted soya beans as an animal feed at this time.

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