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Quality improvement of sweet-potato (*Ipomoea batatas* L. Lam.) roots as feed by ensilage

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1. Sweet-potato (*Ipomoea batatas* L. Lam.) strips (SPS) mixed with maize powder (CP) in proportions 10:0, 9:1, 8:2, and 7:3 were ensiled for 1, 2 or 3 months.

2. Trypsin inhibitor activity (TIA) decreased during ensilage in samples of all treatments while the SPS–CP mixture (7:3, w/w) ensiled for 3 months contained the lowest TIA.

3. SPS–CP (8:2, w/w) dried or ensiled for 2 months, or ensiled for 2 months and dried, were each mixed with twice the amount of control diet (1:2, w/w) to make three diets. These three diets together with the control diet were used for a feeding experiment with rats to evaluate the nutritive value.

4. General composition analysis (including metabolizable energy), fatty acid composition and amino acid analysis (including percentage of essential amino acids) of the samples did not change during ensilage to an extent which could explain the improved performance of rats fed on ensiled diets.

5. Rats fed on diets containing dried SPS–CP (8:2, w/w) showed significantly lower ($P < 0.05$) body-weight gain than rats fed on the control diet or ensiled SPS diets, at the end of the 8th week. They also showed enlargement of the pancreas. The adverse effect of SPS was associated with TIA which seemed to be prevented to some extent by ensilage.

6. The possibility that the starch of SPS may also contribute to the adverse effect cannot be excluded at present.

The presence of trypsin inhibitors (TI) in sweet potato (*Ipomoea batatas* L. Lam.) was first reported by Sohonie & Bhandarker (1954). In feeding experiments with guinea-pigs, the results indicated that the low protease levels resulting from the ingestion of TI in raw sweet potato in conjunction with a low-protein diet allows the β-toxin of *Clostridium welchii* Type C to initiate the disease enteritis necroticans in animals. At normal protease levels, this toxin would be destroyed before there was any intestinal damage (Lawrence & Walker, 1976; Lawrence & Cooke, 1980). Enteritis necroticans is endemic to the Highlands of Papua New Guinea, where until recently it was the main cause of death of children over 1 year of age, and the disease is caused by *C. perfringens* type C (Lawrence *et al.* 1979). Lawrence *et al.* (1979) suggested that the presence of appreciable levels of TI in sweet potato, which constitutes the major component of the diet of the Highland people, may retard the tryptic breakdown of the necrotizing β-toxin produced by *C. perfringens* type C. However, Bradbury *et al.* (1984) reported that the low and high incidences of enteritis necroticans in Erave and the upper Mendi respectively are not due to the difference in content of TI between the sweet-potato cultivars grown in these two regions.

One of the major uses of sweet-potato roots in Taiwan is as feed for pigs. When uncooked sweet-potato roots exceeded 250–300 g/kg diet for pigs, slower growth and a lower protein efficiency ratio (PER) were found (Yeh, 1983), whilst subjecting sweet-potato

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(cv. Tainong 57) strips (SPS) to 588–784 kPa pressure at 164–175° (i.e. popping) raised the starch availability and eliminated TI activity (TIA) completely. At the same time, the growth rates and feed conversion efficiency (FCE) of pigs fed on popped SPS were significantly ($P < 0.05$) improved compared with those of pigs fed on untreated SPS (Yeh *et al.* 1978). The popping process is not practical for general use but it suggests that the cause of the adverse effect of raw sweet-potato roots may be due to TI activity (TIA) and the inferior starch quality of sweet-potato roots. However, Huang & Tsai (1982) reported that whilst the antitryptic activity of sweet potato retarded the growth of young rats (age 5–7 weeks), the adverse effect became insignificant as the period of feeding was continued. The objective of the present study was to examine the effect of ensilage of sweet-potato roots on nutritive value and TIA.

MATERIALS AND METHODS

Materials

Sweet potato (cv. Tainong 64) and maize powder (CP) were bought locally. SPS were prepared fresh or dried at 45° overnight immediately after purchasing. No significant change in TIA of SPS was observed before and after drying.

Control diets for rats (Rodent Laboratory Chow 5001) were products of Ralston Purina Co. (St Louis, USA) with ingredients (g/kg) as follows: crude protein (nitrogen × 6·25) not less than 234; crude fat not less than 45; crude fibre not more than 50; ash not more than 73; added minerals not more than 25; N-free extract about 498; water 75. Trypsin (10000–13000 BAEE units/mg) was purchased from Sigma Chemical Co. (St Louis, USA); all other chemicals were of analytical grade.

Ensilage

Mixtures of SPS with CP (w/w) in proportions 10:0, 9:1, 8:2 or 7:3 were placed into plastic bottles (diameter 70 mm; height 110 mm) and pressed to suitable tightness. The bottles were covered with screw caps and sealed with adhesive tape to minimize aerobiosis. They were then stored at room temperature for 1, 2 or 3 months. Once any bottle was opened after silage making, chemical or biochemical analysis was carried out within 2 d.

Experimental design

Four replicates (four bottles) were used for each of the twelve treatments (four formulations of SPS–CP and three time periods (1, 2, 3 months)). Two samples were taken from each bottle and two independent determinations were made for each sample.

General composition analysis

Analytical procedures were as described by Horwitz (1975) for general composition analysis.

Extract for TIA assays. An 8 g portion of each sample was cut into small pieces and homogenized with 5 vol. double-distilled water (v/w) in a Polytron (Kinematica, Switzerland) homogenizer at 4°. The homogenate was filtered through four layers of cheesecloth and centrifuged at 9000 rev/min using a Sorvall RC-2B (Sorvall, Wilmington, USA) with a SS-34 rotor (9770 × g) for 15 min. The supernatant fraction was used directly for TIA assays without dialysis.

TIA assay with casein as substrate. This followed a modification (Lin & Chen, 1980) of the procedure of Kunitz (1947). For each TIA assay three different assays were undertaken. The standard assay was run by adding 0·5 ml double-distilled water and 1·0 ml trypsin

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solution (containing 20 μg trypsin in 0·25 mM-hydrochloric acid) to tubes containing 1·0 ml heated (100°, 15 min) casein solution (20 g/l; in disodium hydrogen phosphate–sodium dihydrogen phosphate buffer, pH 7·6). Proteolytic reaction was allowed to proceed at 37° for 20 min. The solution was then poured into tubes containing 3·0 ml trichloroacetic acid (100 g/l). The precipitate formed was centrifuged after standing for at least 1 h at about 25°. The concentration of trichloroacetic acid-soluble peptides with aromatic amino acids in the supernatant solution was determined by measuring the absorbance of the solution at 280 nm. The control assay was run by preincubating 0·3 ml crude extract and 0·2 ml double-distilled water with 1·0 ml heated casein solution (20 g/l) at 37° for 15 min. Subsequently 1·0 ml double-distilled water was added and the mixture was allowed to stand at 37° for a further 20 min before being poured into 3 ml trichloroacetic acid (100 g/l). The sample assay was performed by preincubating 0·3 ml crude extract and 0·2 ml double-distilled water with 1·0 ml heated casein solution (20 g/l) at 37° for 15 min. Finally 1·0 ml trypsin solution was added and the proteolytic reaction was carried out as for the standard assay.

Determination of water-soluble protein. Protein determinations were performed by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Calculation of TIA. The percentage inhibition was calculated from the formula $[((A_{280}$ of standard $+ A_{280}$ of control$) - A_{280}$ of sample$)/(A_{280}$ of standard$)] \times 100$ %, where A_{280} is the absorbance of the solution at 280 nm, and was converted to μg trypsin inhibited. For example, 60% inhibition corresponded to 20 $\mu g \times 0{\cdot}6$, i.e. 12 μg trypsin inhibited. The specific percentage inhibition was defined as the percentage inhibition/mg water-soluble protein.

Amino acid analysis. Samples were dissolved in 6 M-HCl, sealed in N_2-flushed tubes and hydrolysed at 110° for 24 h. After hydrolysis, HCl was removed by evaporation. The dried samples were dissolved with 0·2 M-sodium citrate (pH 3·25) and amino acid separation was undertaken on a single column of Dionex-D-300 Component System (Sunnyvale, USA). Derivatives of proline were detected at 430 nm and those of the other amino acids at 570 nm.

Percentage of essential amino acids (% EAA). % EAA was calculated as follows: % EAA = %leucine + %isoleucine + %lysine + %methionine + %phenylalanine + %threonine + %valine + %tyrosine. Neither tryptophan nor cysteine were included in the calculation.

Fatty acid composition. Separation of lipid from the aqueous phase of the test samples was carried out according to Folch *et al.* (1957) and fatty acid composition was determined by separation and quantification of the methyl esters of fatty acids using gas–liquid chromatography (Metcalfe & Schmitz, 1961; Metcalfe *et al.* 1966). The following esterification procedure was used. Approximately 15 mg fatty material was added to a screw-capped test tube with a polyethylene seal. The sample was flushed with N_2 to dryness. Methanolic sodium hydroxide (0·5 M; 2 ml) was added to the mixture and heated in a water-bath at 85° until the fat globules were solubilized (about 6 min) followed by cooling in a water-bath to 25°. HCl (0·7 M; 2 ml) in methanol and 3 ml boron trifluoride–methanol were added to the test tube and the mixture was heated to 85° for a further 6 min and then cooled in a water-bath to 25°. Saturated sodium chloride solution (3 ml) and 4 ml redistilled hexane were added to the mixture which was then shaken vigorously for 1 min and allowed to stand for 30 min. The hexane layer of the mixture was transferred to a vial and flushed with N_2 to dryness. Finally the esters were separated by gas–liquid chromatography using a Varian Model 3700 equipped with a flame ionization detector and a packed column of 15% diethylene glycol succinate on Uniport B (80/100 mesh).

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Table 1. *Estimates of trypsin inhibitor activity (TIA) during ensilage of sweet-potato* (Ipomoea batatas *L. Lam*) *strips (SPS)**

(Mean values of TIA. Standard error 1·29, based on the pooled variation between repeat bottles within treatments, df 36. TIA expressed as specific inhibition, i.e. μg trypsin inhibited/mg protein)

SPS–CP (w/w)	Period of ensilage (months)...	1	2	3
10:0		14·6	14·6	7·0
9:1†		14·0	9·7	4·2
8:2†		11·4	8·9	3·9
7:3†		10·6	10·0	3·3

CP, maize powder.

* Specific inhibition of fresh SPS was mean 37·3 (SE 1·68 df 3) μg trypsin inhibited/mg protein.

† Values corrected for dilution of SPS by CP.

Analytical conditions for the separation were: carrier gas N_2 40 ml/min, hydrogen 49 kPa, air 98 kPa, injection temperature 230°. Temperature programme: initial temperature 150°, 4 min, final temperature 210°, 2°/min.

Ensilage for feeding experiment with rats. This was the same as described previously except that SPS–CP was fixed at the 8:2 (w/w) level with a storage time of 2 months. After ensiling, samples were divided into two parts. One was used directly as an ingredient of feeds without drying treatment (diet C) and the other was dried at 45° overnight before being used as an ingredient of feeds (diet D).

Feeding experiment with rats. Weanling male young Sprague-Dawley rats were divided into four groups. Each group (six rats) was fed *ad lib.* on a different diet. Diet A (control diet) was a product of Ralston Purina Co. In diets B, C and D part (1:2, w/w) of the control diet was replaced by dried SPS–CP (8:2, w/w), or ensiled SPS–CP (8:2, w/w), or ensiled and dried SPS–CP (8:2, w/w). Each rat was confined in a wire cage (470 × 260 × 210 mm) with wood shavings as bedding in a ventilated room at 24–26° but without humidity control, with a 10 h light–14 h dark period. Changes in body-weight and feed consumption of each rat were recorded at the end of every week to calculate feed conversion efficiency (body-weight gain/feed intake (g/g), FCE) and protein efficiency ratio (body-weight gained/protein consumed (g/g), PER) of each rat. After 8 weeks the rats were killed and the liver, pancreas, and spleen of each rat were removed and weighed.

Statistical analysis

Statistical analysis was by Duncan's multiple-range test (Duncan, 1955).

RESULTS

Decrease of TIA during ensilage

Table 1 shows the estimates in TIA in samples of various treatments during ensilage. TIA of samples of treatment SPS–CP (7:3, w/w) was the lowest and fell to 9% of that of dried SPS (3·34/37·3 i.e. 9%) after 3 months of storage. Although a decrease in TIA during ensilage was observed for all samples, the decrease was not linear with ensilage time, whilst for all diet formulations, the time-course patterns of TIA decrease were quite similar.

General composition of ensiled SPS

Table 2 shows composition (g/kg) changes of SPS during ensilage. Both water and ash contents for all samples showed little change. There was a general tendency for crude

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Table 2. *Composition (g/kg) of ensiled sweet-potato* (Ipomoea batatas *L. Lam.*) *strips (SPS)*

(Mean values, with a pooled standard error (df 36) for each variate in parentheses. Four repeat bottles were analysed for each treatment)

SPS–CP (w/w)	Period of ensilage (months)	Moisture	Crude protein*	Crude fat	Crude fibre	Ash	Nitrogen-free extract
10:0	1	724	13·3	4·5	10·4	9·7	239
	2	706	10·9	3·8	9·6	8·9	261
	3	695	9·4	3·0	6·8	9·3	277
9:1	1	683	16·0	7·5	12·8	9·4	272
	2	664	13·6	6·9	11·6	9·3	295
	3	667	11·2	5·2	9·8	9·2	283
8:2	1	604	19·2	11·0	15·6	9·8	340
	2	596	17·1	9·5	13·1	10·2	354
	3	597	15·2	8·9	10·2	9·2	360
7:3	1	564	22·8	14·5	18·4	10·2	370
	2	572	21·7	13·9	16·9	10·4	365
	3	541	18·7	12·0	12·7	9·5	406
		(21·9)	(0·07)	(0·11)	(0·17)	(0·09)	(27·1)

CP, maize powder.
* Nitrogen × 6·25.

Table 3. *Change in fatty acid composition (% by weight) during ensilage of a mixture of sweet-potato* (Ipomoea batatas *L. Lam.*) *strips and maize powder (8:2, w/w)*

(Means of duplicate samples)

Fatty acid	Period of ensilage (months)...	0	1	2	3
16:0		22·4	18·4	18·6	15·7
18:0		trace	trace	trace	trace
18:1Δ9-*cis*		26·0	31·9	29·5	20·5
18:2Δ9-*cis*, 12-*cis*		51·6	49·5	46·0	51·0
18:3Δ9-*cis*, 12-*cis*, 15-*cis*		trace	trace	1·7	12·6

protein levels to decrease in all samples during storage and similar changes were observed in both crude fat and crude fibre levels. The only component which showed a tendency to increase during ensilage was N-free extract (NFE).

Change in fatty acid composition during ensilage of SPS

Table 3 shows changes in fatty acid composition during ensilage of SPS. There was a very rapid increase in the concentration of the fatty acids 18:3Δ9-*cis*, 12-*cis*, 15-*cis* while the proportion of 16:0 decreased slowly. The proportion of 18:1Δ9-*cis* appeared to increase first to a plateau and then decrease as storage continued. Changes in 18:2Δ9-*cis*, 12-*cis* were inconsistent.

Amino acid composition of fresh and ensiled SPS

The values are shown in Table 4. The relative SPS–CP content of samples was 8:2 (w/w) and the samples were ensiled for 2 months. All determined amino acids except methionine

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Table 4. *Amino acid composition (g/kg crude protein (nitrogen × 6·25)) of fresh and ensiled mixtures of sweet-potato* (Ipomoea batatas *L. Lam.*) *strips–maize powder (8:2, w/w)*
(Means of duplicate samples)

Amino acid	Fresh	Ensiled (2 months)
Lysine	7·6*	5·1*
Histidine	6·0	3·8
Valine	11·8	9·1
Aspartic acid	22·5	18·8
Threonine	6·6	5·3
Serine	11·5	9·5
Phenylalanine	10·9	8·8
Glycine	13·0	11·0
Alanine	20·6	16·6
Arginine	6·1	5·5
Isoleucine	4·2	3·1
Leucine	22·6	17·5
Tyrosine	6·7	5·4
Proline	20·3	16·9
Glutamic acid	33·3	25·7
Methionine	3·6	3·7

* This value is corrected for the availability of lysine according to Kan *et al.* (1977).

decreased after ensilage. The % EAA for fresh and ensiled samples were 32·5 and 31·7 respectively.

General composition of various diets

Diet A (control diet) was a product of the Ralston Purina Co. whilst in diets B, C, and D part of the control diet was replaced (1:2, w/w) by dried SPS–CP (8:2, w/w), ensiled SPS–CP (8:2, w/w) or ensiled and dried SPS–CP (8:2, w/w). Diet A contained higher levels of crude protein, crude fat, crude fibre and ash than diets B, C and D. The levels of crude protein, crude fat, crude fibre, ash and NFE in diets B and D were similar whilst diet C contained the lowest levels of crude protein, crude fat, crude fibre, ash and NFE and the highest level of water. Diets A, B and D provided about the same level of metabolizable energy whilst that of diet C provided the lowest level (Table 5) where metabolizable energy (kJ/kg) was calculated from the formula $4{\cdot}186 \times (9 \times \text{fat content (g/kg)} + 4 \times \text{protein content (g/kg)} + 4 \times \text{NFE content (g/kg)})$.

Nutritional evaluation of the test diets

Table 6 shows that, as well as overall response values, the whole experimental period was divided into two subperiods: 0–4 weeks (a fast-growing period with a high body-weight gain, FCE and PER) and 4–8 weeks (a slower-growth period with a lower body-weight gain, FCE and PER). Body-weights of rats fed on control or various SPS diets are shown in Table 6 and Fig. 1. Mean values of initial body-weights of six rats of dietary groups A, B, C and D were 71 (SE 4·5), 71 (SE 3·4), 71 (SE 2·6) and 71 (SE 2·2) g respectively. At the end of the 8th week rats of group A, fed on the control diet, were the heaviest while those of group B, fed on the dried SPS–CP containing diet, were the lightest. Ensilage of SPS (diets C and D) significantly improved the growth performance of rats and there was no

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Table 5. *General composition of various diets (g/kg)**

Diet†	Water	Crude protein (N × 6·25)	Crude fat	Crude fibre	Ash	Nitrogen-free extract (NFE)	Energy ‡ (kJ/kg)
A	100	234	45	50	85	498	13950
B	100	180	41	47	64	568	13980
C	251	161	35	39	60	454	11590
D	100	177	39	44	64	576	14070

* Specific trypsin inhibitor activity (μg trypsin inhibited/mg protein) of fresh sweet-potato (*Ipomoea batatas* L. Lam) strips (SPS) and diets A, B, C and D were 37·04, 0, 10·40, 2·54 and 2·54 respectively.

† A, control diet; B, (dried SPS–maize powder (CP) (8:2, w/w)) – control diet (1:2,w/w); C, (ensiled SPS–CP (8:2, w/w)) – control diet (1:2, w/w); D, (ensiled and dried SPS–CP (8:2, w/w)) – control diet (1:2, w/w). For details of diets, see pp. 174 and 176.

‡ Metabolizable energy (kJ/kg) = 4·186 (9 × fat content (g/kg) + 4 × protein content (g/kg) + 4 × NFE content (g/kg)).

Table 6. *Body-weight (g/rat), body-weight gain (g/rat; BG), mean feed consumption (g/rat per week; MFC), mean protein consumption (g/rat per week; MPC), feed conversion efficiency (FCE) and protein efficiency ratio (PER) of rats fed on control or various sweet-potato* (Ipomoea batatas *L. Lam.*) *strip (SPS) diets*

(Mean values, with a pooled standard error (df 20) for each variate)

	Experimental period (weeks)	Diets*				SE
		A	B	C	D	
Body-wt	8	306^{a}	242^{b}	289^{a}	278^{ab}	12·9
BG	0–4	145^{a}	107^{b}	135^{a}	121^{ab}	8·4
	4–8	90	64	83	85	8·0
	0–8	235^{a}	170^{b}	218^{a}	207^{a}	11·2
MFC	0–4	141^{b}	113^{c}	185^{a}	122^{c}	5·7
	4–8	155^{b}	125^{c}	194^{a}	140^{bc}	5·5
	0–8	148^{b}	119^{c}	190^{a}	131^{c}	4·9
MPC	0–4	33·1^{a}	20·4^{c}	29·7^{b}	21·6^{c}	1·13
	4–8	36·3^{a}	22·4^{c}	31·3^{b}	24·7^{c}	1·20
	0–8	34·7^{a}	21·4^{c}	30·5^{b}	23·2^{c}	1·03
FCE†	0–4	0·256^{a}	0·234^{b}	0·182^{c}	0·247^{ab}	0·0069
	4–8	0·143^{a}	0·128^{ab}	0·107^{b}	0·152^{a}	0·0110
	0–8	0·197^{a}	0·179^{b}	0·144^{c}	0·197^{a}	0·0051
PER†	0–4	1·10^{b}	1·30^{a}	1·13^{b}	1·40^{a}	0·037
	4–8	0·61^{b}	0·71^{ab}	0·66^{b}	0·86^{a}	0·060
	0–8	0·84^{c}	1·00^{b}	0·89^{c}	1·11^{a}	0·030

* A, control diet; B, (dried SPS–maize powder (CP) (8:2, w/w)) – control diet (1:2, w/w); C, (ensiled SPS–CP (8:2, w/w)) – control diet (1:2, w/w); D, (ensiled and dried SPS–CP (8:2, w/w)) – control diet (1:2, w/w).

† FCE, body-weight gain/feed intake (g/g); PER, body-weight gain/protein consumed (g/g).

^{a,b,c} Values in the same horizontal row with different superscript letters were significantly different (Duncan's new multiple-range test): $P < 0·05$.

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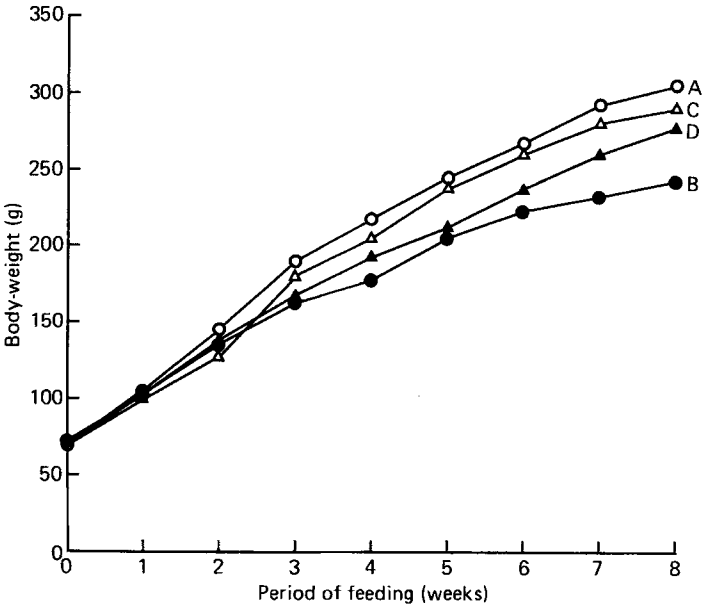

Fig. 1. Body-weight of rats fed on control or various sweet-potato (*Ipomoea batatas* L. Lam.) strip (SPS) diets. A, control diet; B, (dried SPS–maize powder (CP) (8:2, w/w)) – control diet (1:2, w/w); C, (ensiled SPS–CP (8:2, w/w)) – control diet (1:2, w/w); D, (ensiled and dried SPS–CP (8:2, w/w)) – control diet (1:2, w/w). For details of diets, see pp. 174 and 176.

significant difference in either body-weight or body-weight gain between groups A, C and D throughout the whole 8-week feeding period.

Feed intake (g) by each rat was recorded and cumulative feed intakes by the end of the 8th week are given in Table 6.

FCE of group B was lower than those of groups A and D (but only significantly different from that of group A) in the first half-period and was not significantly different from either groups D and A or group C in the second half-period. FCE of group C was the lowest and significantly different from those of groups A, D, and B in the whole period (Table 6). This may be due to the lowest content of nutritional components and energy of the group C diet (Table 5) and the highest amount of feed consumed by rats of group C.

PER of group B was lower than, but not significantly different from, that of group D, but was significantly higher than values for groups C and A in the first half-period. In the second half-period, PER of group B was still between those of groups D and C but not significantly different from both. This subtle change (shifting toward relatively lower efficiency) in both FCE and PER could not be observed in the overall response. Thus, PER of group D was significantly higher than that of group B which was in turn significantly higher than those of groups C and A in the whole period.

All values except mean feed consumption and mean protein consumption in the first half-period were higher than those in the second half-period for all four groups, suggesting a fast-growing period followed by a slower-growing period, as indicated by the *Ad Hoc* Committee on Standards for Nutritional Studies (1977) of the American Institute of Nutrition.

Weights and weight ratios (WR) of organs of rats fed on the control or various SPS diets are presented in Table 7. Weights of pancreas of rats of groups A and B were significantly heavier than those of groups C and D. However, WR of the pancreas of rats of group B

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Table 7. *Weight (W, g) and weight ratio (WR, g/kg body-weight) of organs of rats fed on control or various sweet-potato* (Ipomoea batatas *L. Lam.*) *strip (SPS) diets*
(Mean values, with a pooled standard error (df 20) for each variate in parentheses. Rats were killed after 8 weeks and organs were weighed immediately)

	Pancreas		Liver		Spleen	
Diet*	W	WR	W	WR	W	WR
A	28·0[a]	9·3[b]	114	37·2	5·3[a]	1·8
B	26·5[a]	11·1[a]	96	39·4	4·4[b]	1·8
C	13·8[b]	4·8[c]	111	38·3	5·4[a]	1·9
D	14·4[b]	5·4[c]	107	38·5	5·8[a]	2·1
	(1·36)	(0·59)	(5·8)	(0·76)	(0·24)	(0·08)

* A, control diet; B, (dried SPS–maize powder (CP) (8:2, w/w)) – control diet (1:2, w/w); C, (ensiled SPS–CP (8:2, w/w)) – control diet (1:2, w/w); D, (ensiled and dried SPS–CP (8:2, w/w)) – control diet (1:2, w/w).
[a,b,c.] Values in the same column with different superscript letters were significantly different (Duncan's new multiple-range test; $P < 0·05$).

was significantly the highest of all four groups indicating that enlargement of the pancreas occurred in rats of group B. The feeding of ensiled SPS significantly decreased WR of the pancreas (results of both groups C and D). Weight of spleen of rats of group B was significantly lower than those of other groups. However there was no significant difference in WR between all four groups. This was also true for both weights and WR of liver.

DISCUSSION

The decrease of TIA during the ensilage of SPS was not linear with time (Table 1); TIA fell rapidly to 29·7–39·1 % of the original value (37·3 μg trypsin inhibited/mg protein) after 1 month of storage, remaining at 23·9–39·1 % after 2 months, and finally falling to 9·0–18·8 % after 3 months, suggesting a complicated mechanism. Activity staining (*N*-acetyl-DL-phenylalanine-β-naphthyl ester as substrate of trypsin) and protein staining by polyacrylamide gel electrophoresis have shown at least six protein bands of TI of sweet-potato roots (Dickey & Collins, 1984; Lin & Chu, 1987). The molecular weights of these protein molecules were estimated to be 40400, 39600, 36600, 34800, 22200 and 21500 respectively by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (Lin & Chu, 1987). Ensilage of grass leads to extensive hydrolysis of the plant proteins and to a partial degradation of the amino acids formed (Oshima & McDonald, 1978). Ensilage of leucaena leaves reduces mimosine content and toxicity of samples as feed, suggesting that ensilage leads to a partial degradation of the amino acid (mimosine) (Lin *et al.* 1985). Crude protein, crude fat and crude fibre all decreased while NFE increased during ensilage (Table 2). This suggests that (1) partial or extensive hydrolysis of the proteins of sweet-potato roots and a partial degradation of the amino acids formed may also have occurred during ensilage of SPS, (2) increased NFE may be carbohydrates of small molecular weight which were formed by deamination and reduction of proteins under anaerobic conditions and may give a higher value in the starch availability test than untreated SPS.

Since TI may be the storage form of sweet-potato proteins (Lin & Chen, 1980; Bouwkamp *et al.* 1985) and TIA depends on its precise three-dimensional structure (Laskowski & Kato, 1980) any partial or extensive hydrolysis during ensilage which destroys or distorts an inhibitor's proper structure reduces TIA. Utilization of TI proteins for budding was observed during germination of sweet-potato roots (Y.-H. Lin,

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unpublished results), however, anaerobic microbial fermentation is probably the main process of hydrolysis of TI proteins during ensilage of SPS. Increasing N:carbon favoured microbial growth and, therefore, TIA reduction (Table 1). During the 1st month, TI on the surface of SPS were utilized by microbes, whilst those inside SPS were not utilized until the 3rd month. Alternatively, rapid growth of microbes during the 1st month reduced TIA rapidly, a slow-growth period followed and, finally, during the last month, release of hydrolytic enzymes from dead microbes reduced TIA further.

The large SEM observed in Table 1 is probably not due to another protease present in the SPS extract as suggested by Bradbury *et al.* (1984) who used *N*-tosyl-L-arginine methyl ester as a specific substrate for trypsin. The reason is that although we used casein as the substrate for trypsin, we did not observe any detectable protease activity in SPS extract by the spectrophotometric method. Further, if there were any protease activity in SPS extract, it would have been cancelled out in our procedure of assaying and calculating TIA. Consequently, the large SEM observed is probably due to the fact that ensilage was performed in a somewhat solid mixture of SPS and CP which was not a complete homogeneous system and the growth of microbes was not under strictly-controlled conditions. Hence, sampling from any bottle is bound to give a higher level of errors with values of coefficient of variance (CV) larger than 10%. When crude extract of the same sample was used for TIA assays, CV $\leqslant$ 10% occurred, which may reflect the uncertainty of molecular weight cutoff of protein molecules which are soluble in 50–100 g trichloroacetic acid/l.

Although the TIA of samples of SPS–CP (7:3, w/w) were the lowest, there was no significant difference in TIA levels between samples of SPS–CP (7:3, w/w), SPS–CP (8:2, w/w) and SPS–CP (9:1, w/w) after 3 months of storage. The decision to use the SPS–CP mixture (8:2, w/w) ensiled for 2 months for the feeding experiment with rats was made for two practical reasons: (1) a higher percentage of SPS is better for the utilization of sweet-potato roots, (2) farmers prefer a shorter rather than a longer period for ensilage.

Although diets of the four groups were not isoproteinous, crude protein might not be the limiting factor for any diet when compared with the traditional 100 g protein/kg level (Miller & Bender, 1955; McLaughlan, 1972). The energy contributions by crude protein to the total metabolizable energy of diets A, B, C and D (%) were 28, 22, 23 and 21 respectively.

The improvement of SPS quality as feed by ensilage was obvious (Table 6). However, changes in chemical composition including metabolizable energy (Tables 2 and 5), fatty acids (Table 3) and amino acid composition including % EAA (Table 4) all showed ensiled SPS to be of equal or lower nutritional value than fresh SPS. Hence the improvement of SPS quality as feed when offered in the ensiled form to rats was probably related to the decrease in TIA. A previous study has shown that alkaline treatment, but not heating at 100° or below, diminishes ovomucoid activity, which is heat stable, of raw duck egg white (DEW). This treatment also abolishes growth inhibition of rats by raw DEW, while the amino acid composition of treated DEW is equal or inferior to that of raw DEW (Hsu *et al.* 1979).

The adverse effect of TIA is mild so that although the body-weight gain of group B was lower than those of groups A, C and D, and significantly different from groups A and C in the first half-period (Table 6), no significant difference in body-weight of all four groups was observed in the same period (Fig. 1).

The difference between our results and those of Huang & Tsai (1982) may be due to the formulation of the diets. Since the toxicity of TIA to rats seems to be chronic and mild, a sufficient and constant level of TIA in the diet is required to cause an obvious adverse effect. If we increase the relative amount of normal diet ingredients in groups B, C and D to the

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level at which their nutritive values are close to that of group A, we may observe the same growth performance for all four groups. On the contrary, if we increase the relative amount of SPS–CP ingredient (ensiled or not) mixed with the control diet to more than 1:2 (w/w) (we do not know the exact upper limit yet) the growth performance of rats of groups B, C and D may appear to be the same and inferior to that of group A because the lower protein content of sweet-potato roots (Huang *et al.* 1979) may render dried (not ensiled) SPS and ensiled SPS indistinguishable as a feed ingredient (the effect of insufficient protein quantity may overshadow the adverse effect of TIA). Hence, including SPS–CP ingredient (ensiled or not) at 1:2 (w/w) in diets of groups B, C and D may be critical for observing the adverse effect of dried SPS and improvement of SPS quality by ensilage.

Pancreatic enlargement in the rat caused by diets containing raw soya-bean meal has been well documented (Crass & Morgan, 1982). Results (Table 7) clearly indicate that raw SPS also caused pancreatic enlargement in the rat whilst ensilage of raw SPS prevented this effect. Thus it would appear that improvement in SPS quality as a feed by ensilage must be related to the decrease in TIA (Table 1). The difference between our results and those of Huang & Tsai (1982) might be due to the fact that they compared only the weight of the organs whilst we compared both the weight and weight ratio of the organs. To our knowledge, this is the first report showing that both growth retardation and pancreatic enlargement may be caused by the TIA of sweet-potato roots.

Although rats of group B ate less feed (Table 6), ingested feed and protein were metabolized quite efficiently (FCE and PER, Table 6) at the expense of enlargement of the pancreas. Although FCE and PER for all four groups were lower in the second half-period, the tendency of group B to show a decrease reflected the lesion caused by the cumulative effect of TIA.

On the other hand, although the growth of rats of group C was as good as that of groups A and D, FCE of group C was lower than those of groups A and D at 8 weeks. PER of group C was not significantly different from that of group A but was lower than that of group D at 8 weeks (Table 6). Since the only difference between diets C and D was the inclusion of ensiled SPS–CP in the diet (1:2, w/w) in the former and ensiled and dried (to remove water) SPS–CP in the diet (1:2, w/w) in the latter, the observed differences in both FCE and PER could be explained on the basis that rats of group C ate the most feed, and diet C contained more moisture and less dry matter when compared with the other diets on the same weight basis. Since FCE and PER are calculated on the basis of g feed intake and g protein consumed respectively, a larger denominator will give a smaller ratio when numerators (body-weight gain of groups C and D) are close.

Comparisons of growth performance, FCE and PER of groups C, D and A indicate that the quality of proteins of sweet-potato roots was as good as the control diet once the adverse effect of TIA was removed. This seems to support the results of Huang *et al.* (1979) who showed that although proteins of cooked sweet-potato roots are insufficient to support the normal growth requirement of teenagers and adults, their quality is good.

The brief report of Yeh *et al.* (1978) correlates the improvement of growth rate and FCE of pigs with the improvement of starch availability and elimination of TIA of SPS. Since we did not study changes in the nutritive value of starch and in the structure of starch granules of SP before and after ensilage, the possibility that starch of sweet-potato roots may lower its nutritional quality as a feed cannot be excluded at present.

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