Neurotoxins in a Vetch Food: Stability to Cooking and Removal of γ-Glutamyl-β-cyanoalanine and β-Cyanoalanine and Acute Toxicity from Common Vetch (*Vicia sativa* L.) Legumes

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Because the neurotoxic common vetch (*Vicia sativa* L.) legume seems to have come into some use in man's diet, we have investigated, using Pico-Tag analysis, the stability of the vetch neurotoxins to cooking. When heated in water at 100 °C for 3 h, γ-glutamyl-β-cyanoalanine (γ-gluBCA) cyclized extensively to form pyroglutamic acid and β-cyanoalanine (BCA). By contrast three specimens of common vetch seeds containing 0.42−0.74% γ-gluBCA and 0.01−0.03% BCA retained these principles without loss. A modified cooking procedure replacing the broth during cooking with fresh water and washing the seeds well yielded cooked seeds without detectable neurotoxins. A nutritional study confirmed that the toxins responsible for causing weight loss and mortality in chicks were removed. Lengthy steeping in water at room temperature also effectively removed the neurotoxins from dehulled split seeds but incompletely from intact seeds. These procedures improve the quality of common vetch seed as a source of protein for man.

Keywords: *Common vetch; Vicia sativa L.; legumes; nutrition; chicks; food toxins; detoxification; β-cyanoalanine; γ-glutamyl-β-cyanoalanine; pyroglutamic acid*

INTRODUCTION

Seeds of common vetch (*Vicia sativa* L., CVS) have appeared recently on the commercial food market as an inexpensive surrogate of the lentil (*Lens culinaris* L.) to which it bears considerable physical similarity (Tate and Enneking, 1992). However, CVS is highly neurotoxic to the young chick (Harper and Arscott, 1962; Ressler et al., 1963) due to a neurotoxin that is present as dipeptide γ-glutamyl-β-cyanoalanine (γ-gluBCA, **1**) and to a lesser extent as the free amino acid, β-cyanoalanine, (BCA, **2**). In feeding experiments with the chick, the acute toxicity of the seed has been correlated with the total content of BCA, with both forms of BCA having similar acute toxicity on a molar basis (Ressler et al., 1969).

In the interest of evaluating the suitability of CVS as a source of food for humans, we have examined the stability of the neurotoxins under ordinary conditions of cooking. The stability of purified BCA and γ-gluBCA to the hydrolytic conditions of cooking was examined first. Because other components in the seed, possibly carbohydrate, might influence the stability of the toxins, the effect of cooking on the toxin concentration in the seed was then determined using three separate specimens of CVS. In addition to assessing stability, these experiments furnished information on the ability of several procedures to remove the neurotoxins. These procedures included cooking with several decantations of the broth and steeping in water at room temperature, as well as steeping followed by cooking. To validate the chemical findings that indicated detoxification, the processed cooked vetch was then fed to chicks.

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MATERIALS AND METHODS

BCA was synthetic material (Ressler and Ratzkin, 1961). γ-GluBCA·DCHA had been prepared from material isolated from CVS (Ressler et al., 1969). These had melting points 219−220 °C dec and 184−185.5 °C dec, respectively, close to those reported. γ-GluBCA was a gift from Dr. Max E. Tate. The compounds were homogeneous on TLC, paper electrophoresis, and amino acid analysis (*vide infra*). Pyroglutamic acid and 3,3′,5,5′-tetramethylbenzidine were purchased from Sigma (St. Louis, MO). Splits of specimen **L**, subsequently determined to be *Vicia sativa* (Putnam et al., 1994), originated from seeds reportedly grown in China and imported to Egypt as "red round lentils" (H. Kerien and B. D. Johnson, private communications). Splits of specimen **P** were purchased in 1992 as "masoor dahl" in a local ethnic food store in Minneapolis and were identified as *Vicia sativa* (Putnam et al., 1994). Common vetch seeds, "pea vetch", specimen **C**, were purchased from Seedway, Inc. (York, PA). Grown in Canada, "**C**" was thought to be an improved variety (Willamette) of common vetch but was undocumented (F. S. Mohr, Jr., private communication). One-day old White Leghorn chicks of unspecified sex were obtained from Spafas, Inc. (Preston, CT).

Analyses for protein, fat and fiber were performed by Woodson-Tenent Laboratories, Inc. (Memphis, TN).

Extraction and Amino Acid Analysis (AAA). Extraction of pulverized seed was carried out with 30% EtOH in the cold overnight as described (E-1; Ressler et al., 1969) or modified (E-2) by extracting twice with 20 vol of 30% EtOH with centrifuging and washing, each time with 20 vol of solvent. After a third extraction, the residue was removed by filtration and washed well. The combined extract and washings were concentrated and taken up in water (15 mL/g of seed). Extracts were stored frozen until analyzed. Before derivatization the solution was clarified by centrifugation and an aliquot was ultrafiltered.

AAA was by Pico-Tag analysis involving pre-column derivatization with phenyl isothiocyanate (PITC) followed by HPLC (Cohen et al., 1989). The sample was converted to its base with triethylamine (NEt_3), subsequently treated with 10% PITC-NEt_3 in aqueous methanol for 20 min, and evacuated finally at <100 mTorr for 18 h. Chromatography was on a Rainin 15-cm Microsorb C-18 (5μ) column, using two Waters

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Table 1. Stability to Cooking of γ-Glutamyl-β-cyanoalanine and β-Cyanoalanine in Three Specimens of Seeds of Common Vetch (*Vicia sativa* L.) and of Added γ-GluBCA

	raw[a]		cooked (**A**)[b]				
			γ-gluBCA (%)			recovery	broth content
specimen	BCA (%)[c]	γ-gluBCA	solid	broth	total	(%)	(%)[d]
C seeds[e]	0.03	0.74 ± 0.01	0.22	0.47	0.69	93	52
L splits[e]	0.02	0.42 ± 0.03	0.09	0.31	0.40	95	67
P splits[e]	0.01	0.66 ± 0.05	0.26	0.40[f]	0.66	100	60
P splits[g]		0.71 ± 0.02			0.67	94	
P splits[g] + γ-gluBCA, cooked		1.06			0.91	86	

[a] Based on duplicate extractions, derivatizations, and HPLC analyses. [b] Based on duplicate derivatizations with SE of <±2.8%. [c] For **C**, **L**, **P**, SE are ±5.6, ±4.1, ±1.4%, respectively. [d] Of moist cooked specimens as determined by water content by dry wt of solid in procedure **A**. [e] Specimens were cooked by simmering with 10 parts of water for 3 h and processed as described for **A**. Values from Pico-Tag analysis are based on initial wt of starting specimen. [f] BCA content was 0.03% ± 4.7%. [g] Splits, 0.5 g, cooked in 4 mL of water containing 7.5 μmol of added Nle ± 7.5 μmol of added γ-gluBCA. Cooked mixtures were adjusted to 10 mL of 30% EtOH for extraction. These experiments were carried out simultaneously with Nle recoveries close to quantitative.

M 6000 pumps and a 660 solvent controller. In analysis of raw seeds and splits for γ-gluBCA and BCA (Table 1) extraction and derivatization were monitored with norleucine (Nle) as the internal standard (IS). Subsequently because of the much lower content of BCA, 6-aminocaproic acid was added as a second IS before derivatization. Extractions, derivatizations, and HPLC analyses were performed in duplicate.

For γ-gluBCA in plant extracts the gradient (concave) was 6−60% acetonitrile in 0.14 M sodium acetate buffer containing 0.05% NEt_3 and 0.3 ppm EDTA, pH 6.4 (Cohen et al., 1989), over 60 min so that 50% mixing was attained at 48 min (system 1), flow rate 1 mL/min: R_t γ-gluBCA, 4.2 min; BCA, 17 min; Nle, 43 min. Derivatized BCA was better separated in a similar gradient of 0−50% of 60% acetonitrile in the sodium acetate buffer (system 2): R_t BCA, 43.6 min; ACA 49.2 min. For stability studies of BCA and γ-gluBCA the gradient was 0−60% acetonitrile in 12.5 mM sodium phosphate buffer, pH 6.8, over 24 min (Furst et al., 1990) so that 50% mixing was attained at 17 min (system 3); R_t γ-gluBCA, 7.4 min; BCA, 14.8 min; Nle, 18.4 min. Derivatizations were usually in duplicate.

Ultrafiltration was carried out with Ultrafree-MC low protein-binding regenerated cellulose filter units (10 000 Da cutoff, Millipore Corp., Bedford, MA).

TLC was carried out on Eastman Chromagram polymer-backed cellulose strips in system **4** (1-butanol−pyridine−acetic acid−water, 15:10:3:12 vol). Detection was by spraying with 0.1% ninhydrin in acetone and/or a modified Reindel−Hoppe reagent (Allen, 1989): exposure to chlorine vapor for 10 min, aeration, followed by spraying with 0.1% 3,3′,5,5′-tetramethylbenzidine, in place of the carcinogenic *o*-tolidine, in ethanol.

Electrophoresis was carried out on Whatman no. 1 paper at 10 V/cm for 4.7 h in 0.1 M sodium acetate buffer, pH 5.6 (system 5).

Stability was determined by heating 0.2 mL of 4.2 mM BCA or γ-gluBCA·DCHA and 20 mM γ-gluBCA in sealed 2-mL glass ampules in an oven held at 100−102 °C for 1, 2, and 3 h. The unheated sample served as t_o. Solutions were kept frozen until analyzed. Nle was then added as IS, and the solutions were processed for Pico-Tag analysis.

Seed Processing Procedures. Steeping in Water before Cooking. Common vetch **P** and **C** splits, 5 g, were covered with 20 parts of tap water and allowed to stand at room temperature with occasional mixing. The water was decanted and replaced at least once daily for 8 days. The splits were then collected and dried to constant wt: 3.6 g (72%) and 3.4 g (68%), respectively. A portion was then pulverized, extracted with 30% EtOH, and analyzed. Another portion was cooked. For **P** splits procedure **A** was used; for **C** splits, procedure **B** was used. Intact seeds of common vetch **C** steeped similarly yielded, after removal of 0.66 g (13%) of hard seeds, 3.4 g (68%) that were extracted and analyzed.

Cooking for analysis was carried out in two ways. In **A** the cooked material was separated into solid and broth that were each analyzed separately for BCA and γ-gluBCA, the broth directly and the solid after extraction with 30% EtOH. In **B** the seeds were cooked and processed as in **A** except that after 1, 2, 2.5, and 3 h the broth was replaced by hot water. The broths were combined for analysis. In large-scale processing (**B-1**) only the cooked seeds were collected and analyzed.

A. Two-gram samples of common vetch seeds in 20 mL of water in open 50-mL beakers were kept at a gentle boil on a hot plate. Water was added as needed to maintain the volume. At the end of 3 h the mixtures were passed through mesh strainers. The retained solids were rinsed with water. The solids were weighed, pressed into a paste, and suspended in aqueous EtOH adjusted to give a final volume of 10 mL of 30% EtOH. Extraction was as described (E-1), and the extract was subjected to amino acid analysis (AAA). The broths and rinses were combined and lyophilized to dryness, taken up in water, clarified by centrifugation, and subjected to AAA.

B-1. Processing for Nutritional Use. Intact common vetch seeds, specimen **C**, 1.2 kg, were suspended in 4.8 L of boiling tap water in a stainless steel 8-qt pot. The mixture was boiled gently over a gas flame while the volume was maintained. After 1 h it was poured in portions into two plastic colanders. The seeds were washed well with hot tap water (62 °C) and then returned to the pot. Hot water was added, and the process was repeated for 1 h, then twice at half-hour intervals. The collected moist cooked seeds were then transferred to two 35 × 18 × 1 inch stainless steel trays, blotted well with paper towels, and allowed to dry in a forced draft of air (chemical hood) for 1 week; wt 657 g (55%). The processed vetch contained 22.35% protein, 1.22% fat, 7.9% fiber; 8−9% moisture; 0.006% γ-gluBCA and <0.004% BCA.

Subsequently the cooking/decantation procedure was simplified: On a 2-g scale a single 2-h cooking period followed by copious washing in a strainer with hot water was sufficient to decrease γ-gluBCA from 0.74% ± 0.01 to 0.04% ± 0.001. Cooking for 90 min, washing, followed by cooking for an additional 30 min and washing, resulted in <0.01% ± 0.001 γ-gluBCA. In response to a reviewer's comment about the desirability of conserving fuel and water, the process was shortened further. Steeping for 10 h, followed by cooking for 45 min with decantation and washing at 20 and 45 min, led to no detectable γ-gluBCA in splits and <0.03% γ-gluBCA in intact seeds (Table 2).

Nutritional Evaluation of the Toxicity of Processed Common Vetch Seed. Chicks were maintained under continuous illumination on a commercial, balanced ration Agway Country Feeds Chicken Grower containing 14.5% protein, 3.0% fat, 4.0% fiber (basal diet). Methionine and cystine contents were 0.26% and 0.22%. After 4 days the chicks were matched by weight and placed into 3 groups. The rations were raw vetch/basal (1:1); processed vetch/basal (1:1); and basal. Food and water were taken *ad libitum*. Food consumption and body weight gain were measured usually every 3 or 4 days. The ratio was used to express the efficiency of utilization.

RESULTS AND DISCUSSION

Behavior of Reference γ-GluBCA and BCA under Hydrolytic Conditions. Figure 1 presents the kinetics of decomposition, as determined by Pico-Tag

Table 2. Removal of γ-Glutamyl-β-cyanoalanine and β-Cyanoalanine from Common Vetch Seeds by Cooking/Decantation and Steeping Procedures[a,b]

	raw		processed			
			γ-gluBCA		BCA (%)	
procedure	BCA (%)	γ-gluBCA (%)	solid	broth	solid	broth
specimen **P** splits						
no treatment	0.01[c]	0.66 ± 0.05[c]	–	–	–	–
cooking (**A**)[c]			0.26	0.40	nd	0.04
cooking/decantation (**B**)			<0.01[d]	0.63	<0.005[d]	0.03
steeping (**C**)			<0.01[e]	nd	–	–
steeping/cooking			<0.01[d]	<0.01[d]	<0.005[d]	0.006
specimen **C** splits						
no treatment	0.025[c]	0.91 ± 0.1[f]	–	–	–	–
cooking/decantation (**B**)			<0.006[d]	0.88	<0.006[d]	0.036
steeping (**C**)[g]			<0.006[d]		<0.006[d]	
specimen **C** intact seeds						
no treatment	0.02[c]	0.74 ± 0.01[c]	–	–	–	–
cooking/decantation (**B**)			<0.01		nd	
steeping (**C**)			0.25		0.02[d]	
steeping/cooking/decantation[h]			0.03		<0.003[d]	

[a] Splits were cooked by simmering with 10 parts of water for 3 h and processed as described for **A**. In **B** the broth was replaced several times with water during cooking, and the combined broths and solid were analyzed separately. In **C** the seeds were steeped with 20 parts of water with daily changes for 8 days and analyzed before and after cooking using procedure **A**. [b] Values based on duplicate derivatizations in Pico-Tag analysis with SE ± 1.3−11%. [c] Data of Table 1. nd, not determined. [d] Below this limit of detection. [e] Value in dried solid after steeping raw splits in water. [f] Consistent with the value 0.83% based on the peptide concentration in the intact seed and the fractional weight (0.895) of the kernel in the intact seed. [g] Steeping for 10 h, followed by decanting and cooking for 45 min with washing at 20 and 45 min, gave the same results. [h] Conditions as under *g*.

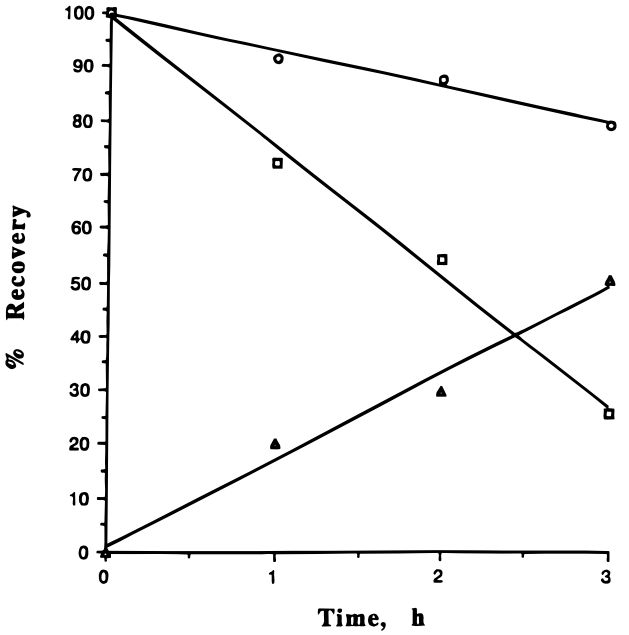


Figure 1. Stability of 20 mM γ-glutamyl-β-cyanoalanine and 4.2 mM β-cyanoalanine in aqueous solution at 100 °C. Values for γ-gluBCA (□) and BCA (○) at t_1, t_2, and t_3 are based on percentage of t_0 values. Values for BCA formed (▲) from γ-gluBCA are based on standards for this amino acid and are corrected for the decomposition of BCA derived from curve ○. SE for (□), <1.2%; (○), <5.8%; (△), <9.6%. Virtually the same results were obtained with 4.2 mM γ-gluBCA.DCHA (not shown) as with γ-gluBCA.

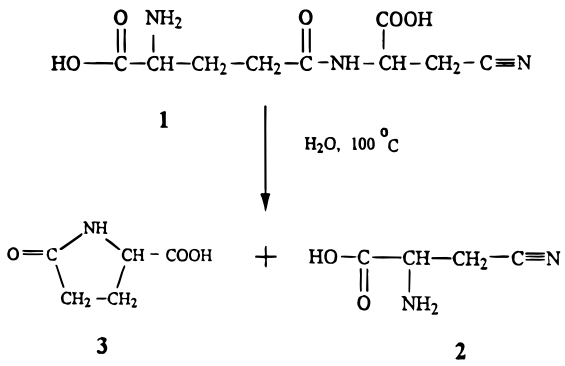
analysis, obtained when γ-gluBCA and BCA in dilute aqueous solution were heated at 100 °C for periods up to 3 h, the hydrolytic conditions of cooking. BCA proved to be quite stable, retaining close to 80% of its starting concentration at the end of 3 h. By contrast, γ-gluBCA, the major form of BCA in CVS, decomposed appreciably with time. After 3 h only 26% remained. Both reactions were first order with $r = 0.99$.

It was of interest that the decomposition of γ-gluBCA was accompanied in part by the formation of free BCA. About 68% of the decomposed peptide appeared as free BCA after 3 h at 100 °C. The formation of BCA from γ-gluBCA was confirmed by TLC by the appearance of material with the mobility and characteristic green ninhydrin color of BCA (Ressler and Ratzkin, 1961) that increased progressively during the course of heating. No other product including glutamic acid, asparagine, or aspartic acid was found by both Pico-Tag analysis and TLC at 3 h. Of the decomposed γ-gluBCA the entire glutamyl residue and 32% of the BCA residue remained unaccounted for. Since both detection methods used depend upon the reactivity of a free amino group, the missing material(s) may lack a free amino group.

Instability of glutamine and α-glutaminylpeptides in aqueous solution at neutral pH has long been recognized. This has been attributed to facile cyclization to pyroglutamic acid (5-oxoproline, **3**; Vickery et al., 1935; Greenstein and Winitz, 1961) or pyroglutamyl peptides, respectively. In structural studies of proteins pyroglutamyl residues occurring naturally or formed artifactually from α-glutaminylpeptides have historically been the basis of difficulties in sequencing and erroneous interpretations. Limited but similar information has been available on the lability near 100 °C of a few γ-glutamylpeptides including glutathione and γ-glutamylcysteine in dilute acid (Binkley et al., 1950) and γ-glutamylglycine and γ-glutamylaspartic acid in water (LeQuesne and Young, 1952; Wieland, 1954). In aqueous hydrolysates of γ-gluBCA the absence of glutamic acid and the formation of BCA suggested that a major portion of the decomposition of γ-gluBCA at 3 h involved cyclization to pyroglutamic acid with liberation of BCA.

The presence of pyroglutamic acid in the 3-h hydrolysate of γ-gluBCA was confirmed by comparing its behavior on TLC and paper electrophoresis with that of authentic *p*glu. A modified Reindel−Hoppe reagent was used for detection. In system **4** *p*glu, R_f 0.46; γ-gluBCA, 0.22; BCA, 0.32; the hydrolysate, R_f 0.46, 0.22, 0.32. In system **5** electrophoretic mobilities were as follows: *p*glu, 19 cm; γ-gluBCA, 13.5 cm; the hydrolysate, 19 cm (yield 55−70%); 13.8 cm; also a small amount of unidentified material, 21 cm. The reaction of γ-gluBCA to give pyroglutamic acid and BCA under

Scheme 1



mild hydrolytic conditions is shown in Scheme 1. It is noted that the first-order kinetics of the decomposition of γ-gluBCA as well as of the formation of BCA (Figure 1) are consistent with the reaction described by the Scheme involving intramolecular cyclization resulting in concurrent displacement of a residue (BCA).

Solid samples of γ-gluBCA·DCHA remained stable at room temperature for 33 years as judged by melting point, paper electrophoresis, and HPLC. γ-GluBCA stored similarly had decomposed extensively, apparently through route(s) other than cyclization. BCA was not detected in the decomposition mixture, and a major product, R_t 3.6 min in system 3, was ninhydrin-positive. This had the electrophoretic mobility (13.7 cm in 1 h in system 5) of hydration product of γ-gluBCA, obtained by treatment of 2.2 mg with a few drops of 35% HBr/acetic acid at 55 °C, and may be γ-gluAsn but was not further investigated. It is clear that reassessing the purity of reference samples of γ-gluBCA may be advisable.

Effect of Cooking Common Vetch Seeds on Their Content of γ-GluBCA and BCA. The specimens of common vetch examined were dehulled splits of Chinese "red round lentils" obtained from an Egyptian importer, dehulled splits of a "masoor dahl" purchased at a local ethnic food store in Minneapolis, and intact seeds of a commercial Canadian common vetch sold for cultivation as well as dehulled splits obtained from the latter (see Materials and Methods). The peptide concentrations were 0.42, 0.66, 0.74, and 0.91%, respectively; BCA ranged from 0.01 to 0.03%. A previous specimen of intact common vetch seeds sold for cultivation contained 0.58% γ-gluBCA and 0.15% BCA (Ressler et al., 1963, 1969). Higher concentrations in other specimens have been reported elsewhere: >1% γ-gluBCA and 1% BCA (Bell and Tirimanna, 1965); 1.1% γ-gluBCA and 0.1% BCA (Tate and Enneking, 1992).

The splits and seeds were cooked in water for 3 h at 100 °C. The cooked solids were separated from the broth and each fraction was analyzed separately for the neurotoxins (procedure **A**). Amino acid analysis showed little change in the total content of γ-gluBCA. The results are shown in Table 1. This stability on cooking held for the three specimens examined, thus affirming that the peptide remained in high concentration after cooking. A similar conclusion was reached independently on cooking specimen **L** (D. H. Putnam, S. Goyal, and W. Breene, private communication of preliminary work).

The contrast between the apparent stability of γ-gluBCA in CVS on cooking and the lability of the purified peptide raised the question whether other components in the seed were stabilizing the peptide. Purified peptide was therefore added to the seed-water mixture before cooking. This resulted in a high recovery (86%) of γ-gluBCA after cooking, thus supporting a stabilization effect of the seed. The results are given in Table 1.

Cooking **P** and **C** splits by procedure **B** increased BCA substantially, e.g., 3- and 1.4-fold (Table 2). However, as the final concentrations were very low, the increases in BCA (<0.02%) were of limited consequence. It may have arisen from <3% decomposition of γ-gluBCA according to the Scheme.

Removal of γ-GluBCA and BCA by Steeping and Modified Cooking Procedures. (a) Steeping. This procedure has long been in use in the home to detoxify cycad kernels (Yang and Michaelson, 1969). Common vetch **P** splits steeped in water at room temperature for 8 days with daily changes of water were analyzed and then cooked and reanalyzed. γ-GluBCA and BCA levels were below the limits of detection of the analyses showing that this mild treatment effectively removed the principles (Table 2, procedure **C**). Results were similar with splits of specimen **C**. With intact seeds of common vetch **C** steeping was less successful presumably due to limited permeability of the seed coats. A portion of the seeds failed to swell and was discarded. When dried, the remaining seeds had one-third the original concentration of γ-gluBCA (Table 2).

(b) Cooking−Decantation. About 60−79% of the peptide recovered after cooking (procedure **A**) was present in the broth with the remainder in the moist cooked seeds. The moisture content indicated that the peptide in the cooked seeds could be attributed to the content of broth (Table 1). This observation suggested that the neurotoxin might be removable by modification of the cooking procedure. The broth was, therefore, replaced by fresh water three times during cooking (procedure **B**). The resulting cooked, washed, moist common vetch **P** splits were essentially free of γ-gluBCA and BCA, their concentrations being below the limits of detection of the analyses (Table 2). Findings were similar with intact common vetch **C** splits and seeds (Table 2). Moreover, the peptide previously present in the raw **P** splits or distributed between the cooked **P** seed and its broth (0.66%, Table 1, procedure **A**) was now confined to the combined broths (0.63%, Table 2). After cooking by procedure **B**, the peptide previously present in the raw splits of **C** (0.91%) was recovered in the combined broth without significant change in concentration (0.88%, Table 2, footnote *f*).

Comparison of the Processed Vetch, Raw Vetch, and Basal Rations for the Chick. In the initial 2−4-day period the chicks' daily food consumptions of the raw vetch/basal, the processed vetch/basal, and the basal rations were in the ratio 0.65:0.79:1. The groups consuming the processed vetch ($n = 6$) and the basal ration ($n = 7$) showed no mortality and excellent growth for the duration of the 16-day experiment. In contrast the groups fed 50% raw vetch ($n = 12$) had a mortality of 50% at 5.9 ± 0.7 days in a 6-day period ($p = 0.05$, Fisher's exact test). Chicks died with the opisthotonus characteristic of β-cyanoalanine (4/6) (Ressler et al., 1967). Weight loss usually preceded death (5/6). The remaining chicks similarly began to lose weight (6/6) and were sacrificed according to required institutional protocol. The average growth curves on the three rations are shown in Figure 2. The cooking/decantation procedure removed the toxins responsible for the weight

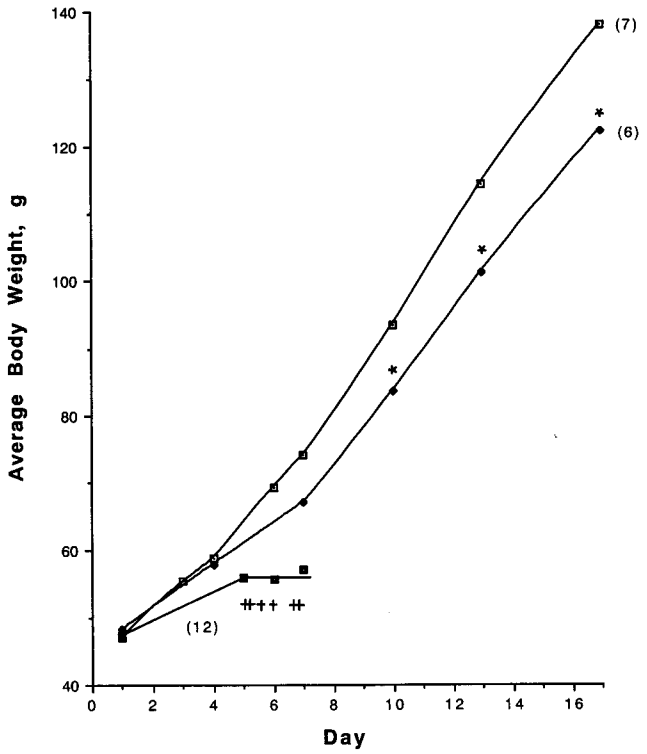


Figure 2. Average growth curves of groups of chicks on three rations: (⊡) basal: commercial balanced feed, 14.4% protein; (◆) 50% processed common vetch seed/basal (1:1); (■) raw common vetch seeds/basal (1:1); +, died; *, group means of body weights for the processed vetch and basal rations were compared using a one-tailed *t*-test. For days 10 and 17, results were statistically significant at $p < 0.05$; for day 13, at $p < 0.025$. By the end of the 16-day period, the basal and processed vetch/basal groups had ingested 255.4 and 267.8 g of diet and gained 90.7 ± 13.8 g and 74.2 ± 10 g per chick, with efficiency of utilizations 2.8 and 3.6 g, respectively.

loss and mortality to chicks. The nutritional findings are consistent with the results of amino acid analysis showing the removal of γ-glutamyl-β-cyanoalanine and β-cyanoalanine by the processing procedure.

Comparison of the Processed Vetch and Basal Rations for the Chick. The food consumption, body weight gain, and efficiency of utilization of the chicks on the three rations are given in the legend of Figure 2. The total food consumption of the processed vetch/basal (1:1) ration was remarkably similar to that of the 100% basal ration alone. Although quite good, the utilization of the processed vetch/basal ration was somewhat less than that of the basal ration. As seen in Figure 2 the processed vetch/basal ration afforded approximately 80% of the growth of the basal ration within the 16-day period. The cooking/decantation procedure used to remove the neurotoxins also removed a large amount of other water soluble substances as reflected in the 45% loss in weight on processing. The protein content of the processed vetch/basal ration was 18.4%, somewhat higher than that of the basal ration (14.4%), suggesting that the amino acid composition of the processed vetch, although adequate, may not be optimal. Supplementation with appropriate amino acid(s) could then prove beneficial.

Common Vetch Seed as a Food. The acute toxicity of raw common vetch seed to the chick is well documented (Harper and Arscott, 1962; Arscott and Harper, 1963; Ressler et al., 1969, and references therein). It is evident also in the present study. Moreover, the toxicity is highly species-specific, with the chick and poult far more sensitive than the rat and pony. There appears to be sparse documentation of episodes of poisoning of man as a result of using *V. sativa* seed as a food. In the Indian culture it is common knowledge that the practice of adulterating other legumes with *V. sativa* is undesirable. However, *V. sativa* cultivar "*blanche fleur*" has recently been developed in Australia as a food export crop (Tate and Enneking, 1992). China also has been exporting significant quantities of *V. sativa* labeled as "red round lentils" (H. Kerien and B. D. Johnson, private communications). Importation of *V. sativa* was recently banned by the Indian government to safeguard the Indian consumer (Bhat and Raghuram, 1993), and commercial exports of common vetch splits leaving Australia are currently labeled as "not for human consumption" (M. E. Tate, private communication). Nevertheless, the distribution of common vetch seeds has caused concern related to the use of inadequate and/or misleading labels when the vetch seeds are redistributed for resale (Bhat and Raghuram, 1993). The presentation of common vetch seed as "lentil" or "masoor dahl" in the food market further complicates the issue of recognizing the etiology of adverse effects of *V. sativa*. Moreover, it is appropriate to consider long-term chronic toxicity resulting from subacute doses of a neurotoxin as well as acute toxicity (Coon, 1973).

This work shows that cooking *per se* cannot be expected to detoxify CVS since it does not lower the concentration of γ-gluBCA, the chief neurotoxin of this seed. This contrasts with the behavior of lectins on cooking certain legumes (Liener, 1981). It is clear, however, that this toxin can be removed readily by suitable processing: common vetch splits may be steeped in water and separated from the liquor before cooking; intact seeds as well as splits can be freed of the neurotoxin during cooking by decanting the broth and washing the seeds well with fresh water. The mortality of common vetch diets for chicks can also be overcome by prolonged autoclaving of the seeds (Harper and Arscott, 1962), a process that destroys free and bound β-cyanoalanine (Washington and Ressler, unpublished data, 1965). Despite the paucity of documented information on the toxicity of common vetch seed for man, it would seem prudent to apply the simple steeping or cooking-decantation technique to remove the neurotoxin before consuming the vetch seed. Since both detoxification procedures remove other water-soluble materials as well, that could include B-vitamins, protein, and carbohydrate, some supplementation of the processed vetch might be indicated if consumed at a high level. Also present in common vetch seeds are a mitogenic lectin (Falasca et al., 1979) and vicine and convicine (Pitz et al., 1980) that have been implicated in favism associated with the consumption of *Vicia faba* (Marquardt, 1989; Brown, 1992). It remains to be determined if these factors are also removed by the processing described.

Suitably processed common vetch seed appears to be a promising source of nutrition for man on the basis of its high protein content, its apparent lack of toxicity, and reasonable efficiency of utilization for the chick, as shown in this work. Moreover, the processing can be carried out conveniently at home. The described nutritional study of processed vetch to chicks was designed primarily to evaluate detoxification. Further investigation of the nutritional quality of processed vetch would be of value. Feeding experiments in chicks using balanced and vetch-containing diets matched for content

of protein and calories have been undertaken (Darre, Minior, Tatake, and Ressler, in progress).

ABBREVIATIONS USED

CVS, common vetch seeds; TLC, thin-layer chromatography; BCA, β-cyanoalanine; γ-gluBCA and γ-gluBCA·DCHA, γ-glutamyl-β-cyanoalanine and its dicyclohexylamine salt; *p*glu, pyroglutamic acid; ACA, 6-aminocaproic acid; NEt_3, triethylamine; IS, internal standard.

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