Nutritional Evaluation of Detoxified and Raw Common Vetch Seed (*Vicia sativa* L.) Using Diets of Broilers†

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This work demonstrates that both processed and raw common vetch seed (*Vicia sativa* L.) can substitute for 10% of the corn−soybean in a balanced diet for 1 month without affecting the growth and feed utilization of young broilers. Two specimens of processed vetch, detoxified by cooking with decantation, contained 22.4 and 25.8% crude protein (N × 6.25). The amino acid composition remained close to that of raw vetch. Processed and raw vetch seeds have relatively high contents of lysine, leucine, arginine, and phenylalanine plus tyrosine and low contents of methionine plus cystine and tryptophan. The lack of toxicity observed for raw vetch is consistent with the dietary level of β-cyanoalanine. Low, but detectable, residues (<0.02%) of this toxin were present in the chicken muscle. The findings are relevant to the potential use of processed vetch for human nutrition and of raw vetch for young poultry.

Keywords: *Processed and raw common vetch seed; Vicia sativa L.; nutrition; broilers; β-cyanoalanine*

INTRODUCTION

The potential to provide an inexpensive additional source of protein has long led to interest in common vetch seed (CVS; *Vicia sativa* L.) for animal, poultry, and human nutrition. Despite knowledge of its toxicity, CVS has appeared recently on the international food market prompted probably by its physical similarity to the lentil (Putnam et al., 1994; Tate and Enneking, 1992).

The 1-day-old chick is particularly sensitive to CVS, making it a good experimental model. The toxicity of CVS to young Leghorn chicks was well-established with respect to mortality and growth retardation at levels of 18−84.8 wt % of the diets (Harper and Arscott, 1962; Arscott and Harper, 1963, 1964; Ressler et al., 1963, 1969, 1997). However, one group of investigators surprisingly observed only growth retardation with 34% CVS in 35-day-old Leghorns (Ocio et al., 1979). More recently, detrimental effects on egg-laying performance and feed intake in hens were found at levels of 7.5−25% CVS (Farran et al., 1995) and 15−46% in corn−soybean-based diets (Castanon and Perez-Lanzac, 1990). CVS contains the toxin β-cyanoalanine (BCA), largely in the form of γ-glutamyl-β-cyanoalanine (γ-gluBCA) (Ressler et al., 1963), which has been correlated with the toxic effect of CVS on the growth and mortality of young chicks (Ressler et al., 1969). Among its actions, this toxin inhibits transsulfuration (Pfeffer and Ressler, 1967), presumably affecting the requirement of sulfur amino acids known to be limiting in legumes (Evans and Bandemer, 1967).

In most of the cited nutritional studies, the concentration of the BCA toxin in CVS was not defined. In commercial samples of CVS the toxin concentration can vary at least 3-fold, and in less available selected varieties it can vary even more widely (Ressler et al., 1969, 1997; and unpublished data, 1995). Moreover, most of the vetch diets were not balanced with respect to the required nutrients. Supplementation with methionine was reported to improve the efficiency of utilization of Chilean common vetch for rats (Araya et al., 1990).

Processing CVS by a cooking−decantation procedure removes both BCA and the acute toxicity for the young chick (Ressler et al., 1997). However, under the conditions studied, the growth response was somewhat less than optimal. The present study was designed to evaluate more carefully the nutritive value of processed vetch. Another purpose of this study was to evaluate the nutritive value of raw CVS for young chicks by taking into consideration the level of BCA contributed by the vetch and the accumulation, if any, of the BCA toxin in the chick tissues, in the form of BCA, γ-gluBCA, and γ-glutamyl-β-cyanoalanylglycine (γ-gluBCAgly) (Sasaoka et al., 1968). Each vetch preparation was incorporated as part of a balanced corn−soybean-based diet that was close to isocaloric and isonitrogenous with the basal broiler diet with which it was compared and contained all essential nutrients according to NRC guidelines (Jurgens, 1988). The nutritional studies required determination of the amino acid composition of the processed and raw vetch.

MATERIALS AND METHODS

Canadian CVS, sample 1, and Oregon CVS, sample 2, were purchased from Seedway (York, PA, and Hall, NY) in 1993 and 1995, respectively. Varieties were thought to be related to Willamette (F. S. Mohr, Jr., private communications, 1993, 1995). Both served as sources for the preparation of the

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detoxified vetch. Sources of γ-gluBCA·DCHA and BCA used as reference are given elsewhere (Ressler et al., 1997).

CVS was processed to remove the β-cyanoalanine toxin using a modification of the method of Ressler et al. (1997). A mixture of 11.45 kg of CVS, sample 2, and 45 L of water was maintained at a gentle boil in a steam-jacketed, stainless steel, covered vessel. At 1 h, 2 h, and 2 h and 20 min the broth was drawn off, the residue was washed and drained, the vessel was refilled with water, and boiling was continued. After another 20 min, the material was filtered, washed, removed, and air-dried in trays in forced-draft laboratory hoods, where it was left for convenience for 9.5 days [wt 6.58 kg (57.5%)]. The product contained no detectable (<0.006%) γ-gluBCA or BCA, determined as described (Ressler et al., 1997).

Fat (ether extract), fiber, energy, protein, and tryptophan contents of raw and processed CVS were determined by Woodson-Tenent Laboratories, Inc. (Memphis, TN) using AOAC methods (Association of Official Analytical Chemists, 1984).

Amino Acid Analysis (AAA). Seed materials were analyzed according to the Pico-Tag procedure (Cohen et al., 1989) after hydrolysis (Ozols, 1990). For Pico-Tag analysis of BCA residues in bird tissues, extracts first were passed through Millipore (Bedford, MA) Ultrafree-MC low protein-binding regenerated cellulose filter units (10 000 Da cutoff). Filtrates and dried hydrolysates were subjected to precolumn derivatization with phenylisothiocyanate (PITC) followed by HPLC. Details are given elsewhere (Ressler et al., 1997). Chromatography was on a 4.6 × 250 mm Rainin (Woburn, MA) Microsorb-MV C-18 (5 μm, 100 A) column with analysis in two systems. System 1 used a concave gradient of 6−60% acetonitrile in 0.14 M sodium acetate buffer containing 0.05% triethylamine and 0.3 ppm of EDTA, pH 6.4, over 40 min so that 50% mixing was attained at 28 min; flow rate = 1 mL/min. Pico-Tag AAA of hydrolysates used system 2, a convex gradient of 6−40% of 60% acetonitrile in the buffer described over 40 min so that 50% mixing was attained at 10 min, as well as system 1.

Diet Formulation and Feeding Trial. Balanced diets containing all essential nutrients in quantities and proportions equal to or exceeding NRC guidelines for growing chicks (Jurgens, 1988a) (Table 1) were calculated with the Brill System computer software (Norcross, GA) for commercial feed formulation at Central Connecticut Farmers' Cooperative Association (Manchester, CT). Raw and processed vetch seeds were incorporated, at 10% of the total, substituting for corn and soybean meal, into corn−soybean-based broiler diets that were as close (<2.5%) to isonitrogenous (∼23% CP) and isocaloric (∼3080 kcal/kg) as possible. The diets were also balanced for methionine (∼0.55%) and methionine plus cystine (∼0.9%).

Sixty 1-day-old male breeder Arbor Acres chicks (Glastonbury, CT) were randomly assigned to one of three dietary treatments: control, raw, and processed vetch. The birds were then divided into two replicates of 10 birds each for each treatment and randomly assigned to six litter-floor pens. The birds were provided free access to water via a bell-type drinker and feed via a gravity feed tube feeder. Heat was supplied with a gas-fired hover. Birds were illuminated with 3.5−5 fc of incandescent light for 23 h per day for the entire period.

The birds were fed the diets for 30 days, during which time the birds and feeders were weighed twice each week, and body weight gain and feed/gain ratios were calculated. At the end of the 30-day treatment period, the birds were killed by electrical stunning, bled out, and processed according to standard methods; carcass weights were determined. Samples of left breast muscle, liver, and kidney were taken, flash-frozen, and analyzed for BCA residues.

Preparation of Tissue Extracts. All operations except extraction with ethyl acetate were carried out in the cold. Frozen tissue (2 g) was homogenized with 10 mL of 5% TCA and 0.2 mL of 20 mM norleucine (Nle), which was used as internal standard. The homogenate was centrifuged for 10 min at 14000*g*. The supernatant was decanted and the pellet resuspended in 10 mL of 5% TCA. Centrifugation and washing were repeated twice. The combined supernatant was then extracted three times with 15 mL of ethyl acetate. The organic extract was backwashed with 3 mL of water. The combined aqueous layer was freed of ethyl acetate by evaporation under reduced pressure. The volume was adjusted to 30 or 35 mL (A).

Table 1. Ingredients and Calculated Analysis of Diets and Composition of CVS

	%, g/100 g of diet		
	control	processed CVS[a]	raw CVS[b]
ingredients			
ground yellow corn (8.6% CP)	55.99	49.82	49.82
soybean meal (49% CP)	33.65	30.35	30.35
vetch	0.00	10.07	10.07
alfalfa meal (17% CP)	2.5	2.52	2.52
meat and bone meal (50% CP)	2.5	2.52	2.52
fat	3.0	2.47	2.47
limestone	1.60	1.51	1.51
salt	0.30	0.30	0.30
methionine	0.21	0.20	0.20
vitamin premix[c]	0.15	0.15	0.15
trace minerals[d]	0.05	0.05	0.05
amprol plus	0.05	0.05	0.05
calculated analysis			
ME, kcal/kg	3082	3006	3011
CP (N × 6.25)	22.98	23.27	23.50
fat	5.79	5.11	5.13
lysine	1.29	1.35	1.36
methionine	0.56	0.55	0.54
methionine + cystine	0.93	0.90	0.90
calcium	1.00	0.95	0.95
available phosphorus	0.25	0.24	0.24
sodium	0.16	0.16	0.16
composition of vetch[e]			
ME, kcal/g		4.0	3.9
CP (N × 6.25)		24.1 ± 2.4[f]	26.4
fat (ether extract)		1.2	0.9
fiber		7.9	4.8
H_2O		9.5	

[a] Mixed product from CVS, samples 1 and 2. [b] Raw CVS, sample 2, containing 0.5% γ-gluBCA + 0.085% BCA (see Materials and Methods). [c] To provide per kilogram of diet: vitamin A, 8,820 IU; cholecalciferol, 2999 IU; vitamin E, 29.76 IU; vitamin K activity, 2.205 mg; vitamin B-12, 22 μg; riboflavin, 8.05 mg; pantothenic acid, 12.13 mg; niacin, 66.15 mg; biotin, 198 μg; thiamin, 2.2 mg; pyridoxine, 4.41 mg; folic acid, 990 μg; ethoxyquin, 132 mg. [d] To provide per kilogram of diet: Mn, 77 mg; Zn, 82.5 mg; Cu, 10 mg; I, 450 μg; Fe, 82.5 mg; S, 10 mg. [e] Corresponding literature values for vetch (*Vicia* spp.) seeds: *ca.* 3 kcal/g, 9.3% H_2O, 29.6% CP, 0.8% ether extract, and 5.7% fiber (*Atlas of Nutritional Data on United States and Canadian Feeds*, 1971). [f] CP of processed samples 1 and 2; other composition data of processed sample 1.

Residue Analysis. The 20 birds fed 10% raw vetch were analyzed in five groups, each containing tissues pooled from four birds, with the sample size 0.5 g for each tissue/bird. Four of the 20 birds fed 10% processed vetch and four of those fed the control ration were each analyzed in two groups of two birds, with the sample size 1 g of tissue/bird. Treated TCA extracts (A) of homogenized frozen tissues of breast muscle, liver, and kidney were analyzed (Sasaoka et al., 1968).

γ-Glutamyl-β-cyanoalanine, γ-gluBCAgly, and BCA in tissue extracts were determined according to the Pico-Tag method with HPLC in system 1: t_R γ-gluBCA, 11 min; t_R γ-gluBCAgly, 13.4 min; t_R BCA, 26.6 min; t_R Nle, 31.8 min. Overall yield in extraction and derivatization was corrected on the basis of the recovery of Nle. Correction was also made on the basis of the stability of γ-gluBCA, which was 63−77% under these conditions. Identity of γ-gluBCAgly in tissues was based on its known accumulation as a metabolite of dietary BCA in tissues of chicks and rats and was confirmed by reduction of the dried

Table 2. Amino Acid Composition of Processed and Raw CVS

amino acid	%, g/100 g of protein	
	processed CVS[a] ($n = 2$)	raw CVS[b] ($n = 2$)
Asp	12.68 ± 0.57	13.39 ± 0.25
Thr	3.39 ± 0.90	2.47 ± 0.22
Ser	4.22 ± 0.93	3.58 ± 0.30
Glu	19.32 ± 0.72	17.85 ± 0.46
Pro	4.85 ± 0.42	4.47 ± 0.11
Gly	4.66 ± 0.03	4.20 ± 0.33
Ala	5.04 ± 0.31	4.80 ± 0.16
$Cys_{1/2}$	1.05 ± 0.05	1.03[c]
Val	5.80 ± 0.18	5.07 ± 0.15
Met	0.87 ± 0.09	0.63 ± 0.14
Ile	4.91 ± 0.38	4.09 ± 0.06
Leu	8.20 ± 0.91	6.60 ± 0.09
Tyr	1.79 ± 0.25	1.53 ± 0.33
Phe	4.99 ± 0.11	4.15 ± 0.45
Trp	1.04[c]	0.93 ± 0.14
Lys	7.25 ± 0.09	6.20 ± 0.18
His	3.39 ± 0.07	3.00 ± 0.10
Arg	6.90 ± 0.34	6.78 ± 0.16
CP[d] (N × 6.25)	24.12 ± 2.43	24.83 ± 2.25

[a] Means ± SEM of products prepared from raw Canadian and Oregon CVS, samples 1 and 2. [b] Means ± SEM of raw CVS, samples 1 and 2, containing 0.75 and 0.5% γ-gluBCA, respectively. [c] Single sample. [d] %, g/100 g of sample.

extract with sodium in liquid ammonia upon which its peak was chromatographically removed and converted to 2,4-diaminobutyric acid after hydrolysis (Sasaoka et al., 1968).

Data Analysis. The broiler growth and feed data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1985). Significant differences between means were determined using Duncan's multiple-range procedures of SAS. Significant differences between means for amino acid data of seeds were analyzed by the Sign test and by a two-group t test. Tissue BCA residue data were analyzed by a one-sample t test.

RESULTS AND DISCUSSION

Protein Content and Amino Acid Composition of Processed and Raw Vetch. The crude protein (CP) contents of Canadian and Oregon raw vetch seed were 23.2 and 26.4%: [reported: 29.5 and 28.5% (Duke, 1981; Fernandez-Figares, 1995); and 24.4 and 23.5% (Castanon and Perez-Lanzac, 1990; Araya et al., 1990)]. Canadian and Oregon vetch after processing had 22.4 and 25.8% CP, reflecting little change in percentage protein despite considerable loss of material. Some material had been lost as a fine pea-soup suspension drawn off and removed with the various broths and washings. The composition of the soluble material lost in the process has not been determined. That some change in the composition of protein took place by fractionation or modification during processing is possible.

The amino acid compositions of raw and processed CVS are given in Table 2. The contents of the essential amino acids generally fell within the ranges reported for raw vetch (Duke, 1981) and were similar for raw and processed vetch. Although the means of 15 of 16 amino acids in processed vetch exceeded those for raw vetch ($p < 0.001$), this was significant ($p < 0.049$) for only 3. Characteristic of both vetch preparations were high contents of Leu, Lys, and Arg and low contents of total sulfur amino acid (TSAA) and Trp.

Effects on Broiler Growth. There was no mortality or morbidity in the three treatments. One bird receiving 10% processed vetch had restricted movement due to tibial dyschondroplasia, and its data were excluded. Otherwise, there were no significant differences ($p > 0.05$) in body weight or weight gain among any of the treatments throughout the trial. The overall feed/gain ratios were also similar, at 1.65, 1.65, and 1.66 for the control and processed and raw vetch diets, respectively (Table 3). Apparently, incorporating 10% raw or processed vetch in a balanced diet had no adverse effect on the feed efficiency and growth up to 30 days.

Evaluation of Raw CVS for Poultry Nutrition. It has been observed that BCA is capable of accumulating in high concentration, for example, as 0.24% γ-gluBCAgly, in chick liver in response to dietary BCA in its free form (Sasaoka et al., 1968). Table 4 gives the residues of BCA in the breast muscle, liver, and kidney of the chicks fed 10% raw vetch for 1 month, in the form of free BCA, γ-gluBCA, and γ-gluBCAgly and as total BCA. As expected, these analytes were not detected in the corresponding tissues of the 10% processed vetch and control groups. Breast muscle from the 10% raw vetch group contained <0.02% total BCA; liver and kidney, <0.05 and 0.06%, respectively. Albeit low in concentration, in liver, γ-gluBCAgly and, in kidney, BCA, were prominent forms, a distribution similar to that observed when BCA was fed to the rat (Ressler et al., 1967). The concentration of total BCA would be <0.004% in the daily diet of a man consuming 1 kg of food containing one chicken breast weighing 200 g.

On the basis of the excellent growth response and feed/gain ratio and the very low level of BCA residue in the chick muscle tissue, raw CVS containing 0.5% or

Table 3. Average Daily Gains and Feed/Gain Ratios of Broilers Fed Processed or Raw Vetch[a,b]

age (days)	av daily gains[c] (ADG; g/day)			feed/gain (g:g)		
	control	processed	raw	control	processed	raw
1						
6	12.0 ± 0.41	12.5 ± 0.40	12.5 ± 0.31	1.60 ± 0.06	1.70 ± 0.12	1.57 ± 0.05
9	21.9 ± 0.70	22.9 ± 0.47	21.9 ± 5.3	1.43 ± 0.00	1.47 ± 0.08	1.42 ± 0.00
13	28.6 ± 0.67	27.9 ± 0.61	28.6 ± 0.60	1.41 ± 0.00	1.42 ± 0.08	1.44 ± 0.02
16	34.6 ± 0.74	34.8 ± 1.24	34.5 ± 0.90	1.57 ± 0.07	1.57 ± 0.00	1.55 ± 0.04
20	41.2 ± 0.80	38.9 ± 1.47	39.3 ± 1.37	1.55 ± 0.01	1.66 ± 0.06	1.60 ± 0.02
23	48.4 ± 1.31	44.8 ± 2.81	46.5 ± 1.93	1.66 ± 0.00	1.86 ± 0.17	1.70 ± 0.06
27	50.5 ± 1.92	47.4 ± 2.18	49.2 ± 2.79	1.83 ± 0.01	1.59 ± 0.27	1.85 ± 0.02
30	52.2 ± 2.72	50.1 ± 3.00	52.6 ± 3.21	2.10 ± 0.09	1.83 ± 0.30	2.14 ± 0.00
av	36.2 ± 1.16	34.9 ± 1.52	35.6 ± 1.46	1.65 ± 0.06	1.65 ± 0.06	1.66 ± 0.06

[a] Means ± SEM. [b] $n = 2$: data from two groups of 10 birds, except for processed vetch data, with one group of 10 and one group of 9 (see text). [c] Initial and final body weights (BW) for control, processed, and raw vetch groups were 50 ± 0.6 and 1063 ± 19.9, 50.4 ± 0.9 and 1027 ± 34.7, and 49.9 ± 1.1 and 1047 ± 33.4, respectively; other BW and growth data available on request. [d] $p < 0.05$ for mean BW and ADG during the 30-day period for the two vetch groups as compared to each other and the control group.

Table 4. BCA Residues in Tissues of 20 Chicks Fed a Balanced Corn−Soybean Diet Incorporating 10% Raw CVS for 1 Month[a]

tissue	%, g/100 g of sample[b]			
	BCA	γ-gluBCA	γ-gluBCAgly	total BCA
breast muscle ($n = 5$)	0.001 ± 0	0.022 ± 0.003	0.015 ± 0.003	0.017 ± 0.003
liver ($n = 5$)	0.002 ± 0.002	0.030 ± 0.007	0.081 ± 0.013	0.045 ± 0.008[c]
kidney ($n = 5$)	0.022 ± 0.006	0.046 ± 0.006	0.038 ± 0.008	0.058 ± 0.012

[a] The diet contained 0.05% γ-gluBCA and <0.01% BCA based on the content of CVS, sample 2. [b,c] Values expressed as means of five groups ± SEM, each group consisting of pooled specimens from four birds. $p < 0.001$ for all values cf. limits of detection of control groups, except for free BCA in liver which is not included in the total BCA for lack of significance.

less of γ-gluBCA as 10% of a balanced high-protein diet thus seems a feasible partial replacement for corn−soybean for the nutrition of young broiler chicks. In developing areas of the world, where corn and soybean are scarce, raw CVS might be a viable substitute in young broiler rations. Whether a higher concentration of vetch in a balanced diet could also be used successfully remains to be determined. Limiting factors in the use of CVS for chick nutrition would be the level of toxin in the vetch sample, the accumulation of BCA in the chick muscle intended for human consumption, and probably the composition of the rest of the diet. Inasmuch as the toxin content depends upon the variety of CVS and possibly the growing conditions, raw vetch should preferably be assayed for toxin when used for food-producing animals. Because in this study the toxin was held below the level known to result in mortality, it is not yet clear whether the high contents of protein and TSAA used have ameliorated any negative effect of the toxin on growth and on the accumulation of BCA residues in tissues.

Evaluation of Processed CVS. Like raw vetch, processed CVS at 10% was fully equivalent to corn−soybean for the growth of young broilers under the conditions studied. Moreover, as compared to their respective controls, growth on the processed vetch diet was significantly better than the 80% observed previously (Ressler et al., 1997). This is attributed largely to the fact that the current diet is balanced. However, differences in the contents of protein (23.5 versus 18.5%) and vetch (10 versus 50%) and in the strain of chick should also be noted as possible factors. Use of processed CVS at dietary levels >10% in a balanced diet may well be feasible. Caloric value and quantity and quality of protein, except for the content of sulfur amino acids and tryptophan, seem satisfactory. Moreover, even when incorporated as 50% of a low-protein, unbalanced diet, processed vetch supported reasonable growth (Ressler et al., 1997). If used as a major part of the diet, however, as with other legumes, supplementation would be appropriate, as, in addition, certain nutrients may be lost on processing.

For economic reasons, processed vetch has greater potential interest for human rather than poultry nutrition. The identity and distribution of CVS in the food market, including the United States, have been well documented (Putnam et al., 1994; Tate and Enneking, 1992). From the data obtained in the previous and current chick trials, vetch seed processed according to the cooking/decantation method so far appears promising for human use. This processing involves only a small modification of the presoaking−cooking procedure already in common use in the home for many beans. Until a variety of CVS with low toxin content becomes more generally available, or one is genetically engineered, it remains appropriate to process CVS intended for human consumption.

ABBREVIATIONS USED

CVS, common vetch seeds; BCA, β-cyanoalanine; γ-gluBCA and γ-gluBCA·DCHA, γ-glutamyl-β-cyanoalanine and its dicyclohexylamine salt; γ-gluBCAgly, γ-glutamyl-β-cyanoalanylglycine; Nle, norleucine; CP, crude protein; ME, metabolizable energy; TSAA, total sulfur amino acid.

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