ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (8): 522-529, 2004

Canavanine Content and Toxicity of Raw and Treated Bitter Vetch (*Vicia ervilia*) Seeds for Broiler Chicken

Gh. Sadeghi*, A. Samie, J. Pourreza and H.R. Rahmani
Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan, P.O. Box 8415683111, Iran
E-mail: ghorbanalis@yahoo.com

Abstract: This study was conducted to determine canavanine content and examine the efficacy of soaking in water, acetic acid or heat treatments on the detoxification of bitter vetch for broiler chickens. A total of 1280 one-day old broiler chicks were placed in 64 pens, twenty in each pen. Treatments were included a corn - soybean based diet as control; raw bitter vetch; soaked in water (1:5, wt/vol) for 12 h, autoclaved (121°C, 20min), then dried at room temperature (SAD); coarsely ground, soaked in water for 24 h, autoclaved and dried (GSAD); coarsely ground, soaked in water for 47 h with exchange of water every 12 h, cooked (75min at 95°C) and dried (GSCD); coarsely ground, soaked at 1% Acetic acid solution for 24h at 60°C (GAAS) bitter vetch in three levels (15, 30, 45%). Each treatment replicated four times. Raw bitter vetch was contained 0.073 percent canavanine. All processing methods reduced canavanine content of seeds to a negotiable amount. Feeding of GSCD and GSAD diet resulted to higher and lower body weight (BW), feed intake (FI) and feed efficiency ratio (FER) at 21, 42 and 49 days, respectively, than other detoxification methods ($P<0.05$). Increasing Bitter vetch level from 15 to 30 and 45 percent significantly declined BW, FI and increased FER ($P<0.05$). Feeding of diet with 15% of GSCD and SAD bitter vetch resulted in performance more similar to control diet ($P>0.05$). Different detoxification methods had no effect on the liver weight, but pancreas weight decreased in all detoxification methods in comparison to raw bitter vetch ($P<0.05$). In all bitter vetch treatments liver weights were higher in 30 and 45% in comparison to15% ($P<0.05$). The results showed that all processing methods were efficient to eliminate canavanine from seeds and GSCD and SAD treatments were more effective to detoxification of the bitter vetch for broiler chicken.

Key words: Bitter vetch (*Vicia ervilia*), detoxification, acetic acid, cooking, autoclave, broiler

Introduction

Throughout the world, many countries are producing seed crops that were adapted to their specific environment and are used as sources of protein in feed for animal or poultry. Some species of leguminous family are sources of cheap protein for animals (Lopez Bellido, 1994). Bitter vetch (*Vicia ervilia*) is known for its high nutritional value, capacity of nitrogen fixation, and ability to grow in poor soils (Lopeze Bellido, 1994). Its seeds contain about 28.5% CP and an AMEn value of 3098 kcal kg^{1} (Farran *et al*., 2001a, b). Bitter vetch seeds has been used in animal feeds and, when treated, as an alternative source of protein in poultry diet (Fernandez-Figares *et al*., 1995; Farran *et al*., 2001b). Raw bitter vetch, however, is detrimental to monogastric animals, especially chickens. The adverse effects arise from the presence of some antinutritional factors in the raw seeds including L-canavanine (0.035-0.11%, Berger *et al*., 2003), trypsin inhibitor (2.14mgg^{-1}DM, Berger *et al*., 2003), Catechin (2.01gkg^{-1}DM, Aletor *et al*., 1994) and a special lectin (Fornestedt and Porath, 1975). Special attention has been paid to the possible role of canavanine in the observed toxic effects. The toxicity of canavanine is probably due to its similarity to that of arginine (D'Mello *et al*., 1990). Canavanine exhibits potent anti-metabolic properties in organism ranging from viruses and prokaryotes to whole animals (Shqueir *et al*., 1989).

Feeding a diet with 60% raw bitter vetch has decreased weight gain and reduced feed intake in broilers and resulted in cessation of egg production of laying hens within 2 weeks post feeding. When soaked in water at 40°C for 72h, it caused an improvement in broiler performance. Soaking the seeds in 1% acetic acid (1:10, wt/vol) at 40°C for 24h was more effective than water treatment in term of performance but was significantly lower than that of control (Halaby *et al*., 1997, personal communication). Farran *et al*. (2001a) showed that raw bitter vetch is an energy rich ingredient but its amino acid availability is low and soaking the seeds in acetic acid at 40°C improved its nutritional value. Ergun *et al*. (1990) showed that addition of 4-12% bitter vetch seeds negatively affected live weight, egg production, feed conversion efficiency and egg weight. Feeding growing rats with raw bitter vetch as a main source of protein over a period of 12 days exhibited a significant reduction in growth, protein efficiency ratio and feed efficiency ratio, as well as, in RNA-activity and RNA/DNA ratio in both muscle and liver (Geona *et al*., 1989). Lasheras *et al*. (1980) experiments showed that oxygen consumption by

intestinal rings increased in chicks fed on bitter vetch diets and in *in vitro* this seed inhibited intestinal transport of D-galactose.

Several detoxification methods have been evaluated for leguminous seeds, including soaking in water (Barbour *et al*., 2001; Farran *et al*., 2001a; Farran *et al*., 2001b; Farran *et al*., 1995; Ologhobo *et al*., 1993), acetic acid (Farran *et al*., 2001a), sodium bicarbonate solutions (Farran *et al*., 2001a) and potassium bicarbonate solution (D'Mello and Walker, 1991), boiling (Dhurandhar and Chang, 1990; Udedie, 1991; Belmar and Morris, 1994; Farran *et al*., 1995), autoclaving (Barbour *et al*, 2001; D'Mello and Walker, 1991), urea treatment (Udedie, 1991), Alkaline extraction (Ologhobo *et al*., 1993), and extrusion (Belmar and morris, 1994; Tepal *et al*., 1994). However, one or more antinutritional substance, representing a relatively high proportion of little-known or unconventional legumes could not be eliminated completely or even partially by the application of the earlier mentioned processing methods and no single processing method appears to be effective in complete removing antinutritional factors. Since most of the protease inhibitors and lectines are thermo labile factors, and L-canavanine is water and acid soluble fraction, the aims of the present work were: (1) investigate the adequacy of multiple treatments (physically and chemically) include grounding, soaking in water and acetic acid at different ways in companion with heat treatments to improve this seed as a feed ingredient for broilers; (2) detection of canavanine in raw and treated bitter vetch to test the hypothesis that the canavanine present in bitter vetch can be responsible for most observed effects.

Materials and Methods

A total of 1280 one-day old broiler chicks of a commercial breed (Aryan) were placed in 64 pens, twenty in each pen. Feed and water were provided *ad libitum*. The chicks were allocated randomly to 16 experimental diets. Treatments were included raw and four different processed bitter vetch seeds in three levels (150, 300, 450 g kg^{-1}) and a corn-soybean based diet as control. Each treatment group consisted of four replicates. Processing methods were included: (1) soaked in water (1:5, wt/vol) at room temperature for 12 h, then autoclaved (121°C, 20min), and dried at room temperature (SAD); (2) coarsely ground, soaked in water for 24 h, autoclaved and dried (GSAD); (3) coarsely ground, soaked in water for 47 h with exchange of water every 12 h, cooked (75 min at 95°C) and dried (GSCD); (4) coarsely ground, soaked at 1% Acetic acid solution for 24h at 60°C and dried (GAAD). The diets (Table 1) were formulated to meet nutrient requirements according to NRC (1994). Diets were isonitrogenous and isocaloric and contain the same levels of methionine, lysine, vitamins and minerals. Crude protein and mtabolizable energy of bitter vetch were determined (26.57 percent and 12.55 Mj Kg^{-1}) by AOAC (1990, 954.1) and Sibbald (1979) methods, respectively, and for amino acid contents used López Bellido (1994) data. The chickens were weighed at the start of the experiment, and during the experiment, live weight and total feed consumption per pen were recorded and feed conversion ratio was calculated at 21, 42 and 49th days of the experiment. Mortality was also recorded for each treatment. Chicks that had allocated to 450gkg^{-1} raw and processed bitter vetch diets had very low body weight and were eliminated from the experiment at day 42. Tow birds from each replicate were slaughtered at days 14, 28 and 42 and liver and pancreas were removed, weighed and presented as a percentage of live weight.

Canavanine content of raw and treated bitter vetches was determined by HPLC using Acamovic and D'Mello (1990) method for gradient condition and Sedgwick *et al*. (1991) for derivitization condition. Seed samples were ground to pass through a 1mm mesh and 0.1 g of samples were subjected to 6 N HCl and hydrolyzed for 24 h at 110°C. Acid hydrolyzed samples were subsequently analyzed for canavanine content by reverse phase high performance liquid chromatography with pre-column o-phhaldldehyde (OPA) derivatization. Canavanine was separated on a 4.6 × 150 mm supelcosil 3 micron LC-18 reverse phase column (Supelco, Oakville, Ontario).

The results obtained from the experiment were analyzed by an analysis of variance using the general linear model (GLM) procedure of SAS and means were compared by Duncans Multiple Range Test (SAS Institute, 1995).

Results and discussion

Canavanine content: Raw bitter vetch seeds contained an average of 0.073 percent canavanine. All processing methods reduced canavanine content. GSCD treatment was most effective method in this case and removed canavanine completely. Other extraction procedures reduced canavanine to a negligible concentration. GAAD treatment decreased canavanine to 0.007 percent and SAD and GSAD treatments reduced canavanine content to 0.01 percent. Berger *et al*. (2003) had reported that *vicia ervilia* seeds contained 0.035 to 0.11 (mean: 0.083) percent canavanine, which is quiet comparable to the levels detected in the present study. Canavanine is a water soluble non amino acid, but it is stable at temperatures of up to 135°C (D'mello and Walker, 1991). These results were showed that soaking in water or acetic acid regardless of temperature can remove canavanine from bitter vetch seeds.

Total feed intake: At the ages of 21,42 and 49-days, chicks received GSCD diet had highest figure than other treatments in term of total feed intake ($P<0.05$) and raw

bitter vetch diet resulted in lower feed intake but its difference from GSAD and GSCD diets was not significant (Table 2). Increasing the level of the bitter vetch from 15 to 30 and 45 percent in all diets decreased feed intake regardless of processing (Table 2). In comparison with control diet chicks fed on diets containing 15 percent of GSCD, GSAD and SAD bitter vetch showed more similar feed intake to control at 21 and 42 days of experiment, and diets containing 30 and 45 percent raw and 45 percent GSAD bitter vetch resulted in lowest ($P<0.05$) feed intake (Table 2). These findings are in agreement with Ocio *et al.* (1980a) results that showed chicks given 15-35% raw bitter vetch seeds consumed less feed and gained weight more slowly. Ocie *et al.* (1980b) and D'Mello (1995) have also reported decrease in feed intake in bitter vetch fed birds. The decrease in feed intake in chicks fed with raw bitter vetch diet could be associated with the presence of antinutritional factors in this legume, specially canavanine and lectin. This response may be the reaction of bird to these toxic factors in the digestive tract or some unpleasant taste caused by these factors in the diet. Michelagel and Vargas (1994) added canavanine (free base or sulfate salt) to a control diet in an amount equivalent to that provided by 300 g raw jack bean/kg diet. Supplemented diets with canavanine depressed feed intake by 30% in relation to the control diet. By contrast, Shqueir *et al.* (1989) found no statistically adverse effect on feed intake and growth rate after feeding chicks a diet containing $0.056 gkg^{-1}$ canavanine for 2 weeks. The mechanism whereby canavanine depressed feed intake in chicks remains to be determined. The most consist evidence of some metabolic disturbance induced by canavanine seems to be the finding that the plasma basic amino acid pattern is severely disarranged. Feeding a diet include free-base canavanine caused a significant depression in concentration of plasma histidine, lysine and arginine compared with control diet (Michelagel and Vargas, 1994). It is now generally accepted that rats and chicks fed disappropriate amount of amino acid or fed excessive or different amounts of an individual amino acid rapidly reduce their feed intakes (Austic, 1986). D'Mello *et al.* (1990) hypothesized that there was a canavanine-arginine interaction in chicks fed on autoclaved jack beans (AJB), paralleling the well-established lysine-arginine antagonism. Lys-Arg antagonism leads to depression in food intake which invariably also accurse in animals fed amino acid imbalance diets (Austic, 1986). The mechanism responsible for this depression in feed intake has yet to be elucidated. However, it has been proposed that changes in brain concentration on neurotransmitters, derived from essential amino acids, may be implicated in the reduction of feed intake (Li and Anderson, 1983). Another possibility for action of canavanine may be associated with its inhibition of nitric oxide formation from arginine (Harbak *et al.*, 1994) and feed intake response may also be regulated by nitric oxide (D'Mello, 1995). In addition, Fornestedt and Porath (1975) have found a new lectin in seed of *vicia ervilia* that its sugar specifity appeared similar to that of concanavaline A and it was rich in acidic and hydroxylic amino acids. Some studies have shown that the rate of stomach emptying appears to be greatly decreased in exposure to some dietary lectins. Since various hormones, particularly cholecystokinin (CCK), have been found to influence feed intake and passage rate of nutrient from the stomach, the rapid change in rate of stomach emptying may suggest that dietary lectins can modulate secretion of CCK and/or other gastrointestinal hormones and thereby affecting stomach emptying and feed intake (Grant, 1989).

GSAD and SAD treatments improved feed intake more that others. Compared with control, diets contained 15 percent SAD and GSCD treated bitter vetch decreased feed intake, but this depression was not as great as with the raw bitter vetch. It is clear that an improvement in the feed intake of chicks resulted from this processing methods is a result of canavanine reduction in theses diets. Also these treatments may be more efficient in removing of other toxin factors from bitter vetch seeds. Lectins are heat sensitive proteins and heat treatment is a good method of decreasing the activity of lectins, and trypsin and chymotrypsin inhibitors, generally improving the nutritional value of grain legumes (Kadam *et al.*, 1987). It seems there are some other antinutritional factors that these water, acetic acid, boiling and autoclaving treatments are not able to remove or destroy.

Live weight: At the ages of 21,42 and 49 days GSCD caused a higher live weight than raw and other processed bitter vetch diets ($P<0.05$), and the lowest ($P<0.05$) live weight at 49-day-old chicks belonged to raw bitter vetch diet (Table 2). Increasing raw and treated bitter vetch diets from 15 to 30 and 45 percent diets decreased body weight in all ages (Table 2). Interaction effects of processing methods and level of bitter vetch in diet are shown in Table 3. Chicks fed on diets containing 15 percent GSCD bitter vetch and 15 percent SAD bitter vetch showed more similar live weight to control group at 21 and 42 day-old birds and lowest live weight was obtained with diets containing 30 and 45 percent raw and 45 percent GSAD bitter vetch ($P<0.05$). This result is in agreement with Santidrian *et al.* (1980) who showed in male white leghorn chicks were grown on diets with raw bitter vetch as main source of protein (21-23%), from one-day old to 7 weeks of age, daily gain was 4.6g compared with 30.1g for control. Michelagel and Vargas (1994) also showed that the weight gain of chicks fed canavanine containing diet was 26% lower than chicks fed control diet. The poor growth found upon feeding of

Table 1: Composition of experimental chicken diets and calculated major components (g kg^{-1} as fed)

Ingredient	1-21 day				21-42 day				42-49 day			
	0	15	30	45	0	15	30	45	0	15	30	45
Corn	573.4	507.2	436.3	366	657.8	589.3	420.4	448.9	680	611	540.9	473
Soybean	318.7	242	167.7	93	264.7	189.2	114	39.3	249.6	174.3	99.4	23.4
Bitter vetch	0	15	30	45	0	15	30	45	0	15	30	45
Fish meal	30	30	30	30	15	15	15	15	0	0	0	0
Vegetable fat	40.5	33	27	21	27.6	21	14.6	9.9	37	30.5	25	18
Oyster shell	14.5	14.5	15	15.1	15.5	15.5	15.1	15	16	15.9	15.5	15.2
Dicalcium phosphate	13	12.5	13	13.5	10.6	10.6	11	11.5	9.7	10.1	10.5	10.5
D-L Methionin	1.7	1.7	1.8	1.85	0.8	0.8	0.8	0.8	0.3	0.5	0.6	0.8
Lys- Hcl	0	0.9	1	1.5	0	0.6	1.1	1.6	0	0.5	0.9	1.5
NaCl	3.2	3.2	3.2	3.2	3	3	3	3	2.6	2.6	2.6	2.6
Mineral premix[a]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[b]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Calculated Composition												
ME (MJ/Kg)	12.73	12.73	12.73	12.73	12.79	12.79	12.79	13.1	13.1	13.1	13.1	13.1
CP (g/Kg)	218.2	218.2	218.2	218.2	191	191	191	191	176	176	176.1	176.2
Met (g/Kg)	5.2	5.1	5.1	5.1	4	3.9	3.9	3.9	3.3	3.3	3.3	3.3
Lys (g/Kg)	12.8	12.8	12.8	12.8	10.5	10.5	10.5	10.5	9.3	9.3	9.3	9.3
AP (g/Kg)	4.4	4.4	4.3	4.3	3.5	3.5	3.5	3.5	2.9	2.9	2.9	2.9
Ca (g/Kg)	9.5	9.5	9.5	9.5	8.7	8.7	8.7	8.7	7.8	7.8	7.8	7.8

[a]supplemented (mg kg^{-1} of diet): Mn, 1200; Fe, 60; Zn, 120; Cu, 12; I, 1.2; Se, 0.24

[b]supplemented (mg or IU kg^{-1} of diet): Vit. A, 10800 IU; D_3, 2400 IU; E, 21.6 IU; K_3, 2.4 IU; B_1, 2.16; B_2,7.9; B_3, 12; B_5, 3.6; B_9, 1.2; B_{12}, 0.015; Biotin, 0.12; choline chloride, 600; and adequate anti oxidant.

Table 2: Effect of feeding raw and treated bitter vetch (GAAD, GSCD, SAD and GSAD) and those different levels inclusion in diet on total feed intake (TFI), live weight (LW), feed conversion ratio (FCR) and mortality

	21-day			42-day			49-day			
	TFI (g)	LW (g)	FCR	TFI (g)	LW (g)	FCR	TFI (g)	LW (g)	FCR	Mortality (%)
Processing										
Raw	521^{d}	216^{dc}	2.41^{a}	1990^{d}	523^{d}	3.80^{a}	3076^{c}	900^{c}	3.42^{a}	1.25
GAAD	604^{c}	221^{c}	2.73^{a}	2088^{c}	573^{c}	3.64^{a}	3243^{c}	916^{c}	3.54^{b}	1.25
GSCD	715^{a}	380^{a}	1.95^{b}	2561^{a}	949^{a}	2.96^{b}	3896^{a}	1403^{a}	2.77^{c}	0.67
SAD	647^{b}	319^{b}	1.88^{b}	2301^{b}	792^{b}	2.70^{b}	3561^{b}	1278^{b}	2.79^{c}	0.75
GSAD	540^{d}	197^{d}	2.74^{a}	1984^{dc}	511^{d}	3.88^{a}	3294^{bc}	867^{c}	3.80^{a}	1.00
Level										
15	755^{a}	398^{a}	1.90^{c}	2692^{a}	1109^{a}	2.42^{c}	3909^{a}	1423^{a}	2.75^{b}	0.75
30	619^{b}	260^{b}	2.38^{b}	2159^{b}	598^{b}	3.61^{b}	2920^{b}	723^{b}	4.03^{a}	0.85
45	442^{c}	145^{c}	3.04^{a}	1650^{c}	301^{c}	5.48^{a}	ND*	ND	ND	1.35^{a-}

[d]Values with no common following letter in each column differ significantly($p<0.05$), *ND: not determind

dietary levels of bitter vetch has been primarily attributed to a feed intake reduction. However, it seems that it is not a major factor in growth inhibition caused by bitter vetch contained diets, For example, broilers fed on 15 percent GSAD diet showed a feed intake similar to control diet, but body weight was significantly ($P<0.05$) lower than control and15 percent SAD and GSCD diets. Growth depression from feeding grain legumes is usually due to more than one factor. Canavaine, Lectine and trypsin inhibitors have been shown to have some antimetabolic activities. The arginine like structure enables canavanine to bind many enzymes that usually interact with arginine and it also incorporates into polypeptide chains, resulting in structurally aberrant canavanine-containing proteins (Rosenthal, 1977). Canavanine may inhibit from arginine uptake in small intestine (Grenson *et al.*, 1966) or L-arginine Na^{+} dependent transport across the enterocyte apical membrane in broiler chickens intestinal brush border membrane vesicles (Rueda *et al.*, 2003). Geona *et al.* (1989) showed that as compared to heated soy bean-fed rats, those fed the legume *Vicia ervilia* diet exhibit a significant reduction in growth and protein synthesis capacity significantly increased in liver but not in the muscle. A significant effect of canavanine treatment is production of canavanyl proteins and the curtailing of RNA metabolism. DNA metabolism and overall protein production are affected secondarily (Rosenthal, 1977).

Table 3: Effect of feeding raw and treated bitter vetch in different levels in comparison to control on total feed intake (TFI), Live weight (LW), feed conversion ratio (FCR) and mortality

Processing	level	21-day			42-day			
		TFI (g)	LW (g)	FCR (g/g)	TFI (g)	LW (g)	FCR (g/g)	Mortality (%)
Control		838^{a}	493^{a}	1.70^{f}	2864^{a}	1640^{a}	1.74^{f}	0.5^{b}
Raw	15	719^{cde}	389^{abcd}	1.85^{ef}	2505^{bc}	1018^{dc}	2.47^{ef}	0.5^{b}
	30	512^{i}	171^{e}	3.00^{c}	1804^{ef}	366^{g}	4.93^{d}	0.6^{b}
	45	331^{j}	90^{e}	3.69^{ab}	1386^{g}	184^{i}	7.53^{a}	3.25^{a}
GAAD	15	684^{def}	340^{dc}	2.02^{edf}	2528^{bc}	947^{d}	2.67^{ef}	1.25^{b}
	30	580^{gh}	353^{bcd}	3.28^{bc}	2041^{dc}	499^{f}	4.10^{d}	1.5^{b}
	45	498^{i}	133^{e}	3.76^{a}	1696^{f}	271^{h}	6.26^{b}	1.2^{b}
GSCD	15	762^{bcd}	451^{ab}	1.69^{f}	2774^{ab}	1288^{b}	2.16^{ef}	0.5^{b}
	30	742^{bc}	405^{abc}	1.84^{edf}	2618^{ab}	995^{dc}	2.16^{ef}	1.25^{b}
	45	641^{fg}	283^{d}	2.27^{d}	2292^{dc}	563^{f}	4.07^{d}	0.5^{b}
SAD	15	791^{ab}	434^{abc}	1.82^{ef}	2817^{a}	1254^{b}	2.25^{ef}	1.25^{b}
	30	672^{cf}	384^{bcd}	1.75^{f}	2307^{e}	778^{e}	2.97^{e}	0.5^{b}
	45	477^{i}	138^{e}	3.47^{abc}	1779^{ef}	343^{gh}	5.19^{c}	0.5^{b}
GSAD	15	836^{a}	372^{bcd}	2.26^{de}	2833^{a}	1038^{c}	2.73^{ef}	0.5^{b}
	30	519^{hi}	136^{e}	3.82a	2025^{e}	352^{gh}	5.75^{bc}	0.75^{b}
	45	261^{k}	84^{e}	3.16^{c}	1092^{h}	144^{l}	7.58^{a}	1.75^{ba-i}

Values with no common following letter in each column differ significantly ($p<0.05$)

In addition, have been shown some lectins cause a morphological change in the small intestine and reduction in the activities of intestinal and brush border enzymes such as entrokinase, leucinaminopeptidase, alkalin phosphatase, maltase and sucrase. A lowering of lipid absorption was also observed (Grant, 1989). These effects reduced the digestive and absorptive capacity of the small intestine and therefore the lectin fed animals lost weight as a result of malabsorption of dietary nutrients. Faran *et al.* (2001a) showed that untreated vetch and bitter vetch are detrimental to amino acid availability and showed that true availability of Lys and Arg was lower than of soybean meal. Larbier (1987) reported a 12% reduction in true digestibility of arginine and lysine in chicks fed Canavalia ensiformis, a seed containing canavanine and lectin. Besides, the presences of trypsin inhibitors have been implicated in reducing protein digestibility (Liener, 1980). Improvement of the body weight in broilers fed on diets containing GSCD and SAD, alongside with improvement in feed intake shows that these treatments are more effective in bitter vetch detoxification, but could not produce a body weight same as control. It is possible that these treatments are not sufficient to eliminate all toxic factors, or these processing methods may lead to some nutrient losses in bitter vetch and resulted to an imbalance bitter vetch containing diets.

Feed conversion ratio: Feed conversion ratio of birds fed on GSCD was lower than other treatments in all ages and raw and GSAD diets resulted in higher ($P<0.05$) feed conversion ratios (Table 2). In all groups, feed conversion ratio at 21 and 42-days was increased ($P<0.05$) by increasing the level of bitter vetch from 15 to 30 and 45 percent (Table 2). Chicks consuming the diet containing15 percent GSCD had similar feed conversion ratio to control at 21 and 42 days ($P>0.05$) and its difference with other treated bitter vetch seeds at 15 percent inclusion was not significant. Feed conversion ratio in 42 day old chicks fed on diets with 45 percent of raw and GSAD bitter vetch was 4.45 times higher ($P<0.05$) than control (Table 3). This result is in agreement with the finding of Ocie *et al.* (1980b) who showed the chickens given 13% raw seed grew more slowly and had higher feed conversion ratio than those given diets without seeds. The results in feed conversion ratio are completely coincident with feed intake and live weight results.

Mortality: Mortality was not significantly different among groups fed raw or different treated diets and in different levels of bitter vetch inclusion (Table 2). The only significant differences ($P<0.05$) in mortality rate was when chicken fed 45% of raw bitter vetch. (Table 4). These results are in agreement with the findings of Ocio *et al.* (1980a) who showed no significant differences in chicks fed on diets containing 18, 25 and 35 percent raw bitter vetch and control. This results showed that antinutritional factors in bitter vetch although has dramatic effects on bird performance, but those amount in diets containing up to 30 percent of raw bitter vetch could not significantly affect mortality rate.

Organs weight: Liver weight at 2 week-old birds was not

Table 4: Liver weight (LW) and pancreas weight (PW) of broilers fed on raw and treated bitter vetch diets and different levels of those inclusions at 2, 4 and 6-weeks of age (as g/100 g live weight)

Processing	2-week		4-week		6-week	
	LW	PW	LW	PW	LW	PW
Raw	4.32	0.58^{a}	3.67^{a}	0.46^{a}	3.31^{a}	0.36
GAAD	3.82	0.43^{b}	3.33^{ab}	0.41^{ab}	3.26^{ab}	0.35
GSCD	3.73	0.49^{b}	2.91^{b}	0.31^{c}	2.65^{ab}	0.30
SAD	4.26	0.46^{b}	3.40^{ab}	0.37^{bc}	2.65^{ab}	0.26
GSAD	4.05	0.46^{b}	3.55^{a}	0.42^{ab}	2.55^{b}	0.29
Level						
15	3.83	0.47	3.00^{b}	0.35^{b}	2.40^{b}	0.26^{b}
30	4.11	0.50	3.40^{a}	0.41^{a}	3.34^{a}	0.46^{a}
45	4.20	0.51	3.71^{a}	0.43^{a}	2.91^{ab}	ND

*$^{a-c}$ Values with no common following letter in each column differ significantly($p<0.05$), *ND: not determined

Table 5: Liver weight (LW) and pancreas weight (PW) of broilers fed on raw and treated bitter vetch diets at different levels in comparison to control at 2, 4 and 6-weeks of age (as g/100 g live weight)

Processing	level	2-week		4-week		6-week	
		LW	PW	LW	PW	LW	PW
control		3.01^{b}	0.4^{bc}	2.44^{c}	0.30^{e}	2.16^{b}	0.20^{b}
Raw	15	3.87^{ab}	0.59^{a}	3.43^{abc}	0.38^{bcde}	2.33^{b}	0.27^{b}
	30	4.74^{ab}	0.60^{a}	2.97^{bc}	0.47^{ab}	3.75^{ab}	0.48^{ab}
	45	4.35^{ab}	0.59^{a}	3.51^{abc}	0.53^{a}	3.12^{ab}	0.34^{b}
GAAD	15	4.29^{ab}	0.44^{abc}	3.02^{bc}	0.37^{bcde}	2.47^{b}	0.24^{b}
	30	3.49^{ab}	0.43^{abc}	3.39^{abc}	0.44^{abcd}	4.26^{a}	0.52^{ab}
	45	3.79^{ab}	0.43^{abc}	3.58^{abc}	0.44^{abcd}	3.04^{ab}	0.28^{b}
GSCD	15	3.79^{ab}	0.48^{abc}	2.70^{c}	0.28^{e}	2.33^{b}	0.24^{b}
	30	3.63^{ab}	0.53^{abc}	2.97^{bc}	0.32^{de}	2.94^{ab}	0.82^{a}
	45	3.81^{ab}	0.46^{abc}	3.06^{bc}	0.34^{dce}	2.69^{ab}	0.27^{b}
SAD	15	3.32^{ab}	0.45^{abc}	2.83^{c}	0.35^{bcde}	2.39^{b}	0.24^{b}
	30	4.38^{ab}	0.46^{abc}	3.26^{abc}	0.37^{bcde}	2.40^{b}	0.26^{b}
	45	5.00^{a}	0.46^{abc}	4.12^{ab}	0.39^{bdec}	2.69^{ab}	0.27^{b}
GSAD	15	3.76^{ab}	0.39^{c}	3.06^{bc}	0.34^{dce}	2.55^{b}	0.30^{b}
	30	4.52^{ab}	0.48^{abc}	3.29^{abc}	0.46^{abc}	2.54^{b}	0.27^{b}
	45	4.25^{ab}	0.50^{abc}	4.29^{a}	0.45^{abcd}	2.54^{b}	0.30

$^{ba-e}$ Values with no common following letter in each column differ significantly ($p<0.05$)

affected by processing method but in 4 and 6-week-old birds fed on GSCD and GSAD diets, respectively, liver weight was significantly ($P<0.05$) higher than control (Table 4). In 2 week-old chicks all processing methods resulted to lower pancreas weight than untreated group, but in 4-week-old chicks GSCD and SAD showed significant difference with untreated group ($P<0.05$). In 6 week old birds no significant differences was observed in liver weight (Table 4). At ages 4 and 6 weeks, increasing bitter vetch levels from 15 to 30 and 45 percent resulted in significant ($P<0.05$) increase in liver and pancreas weight (Table 4). Interaction effect of processing methods and levels of bitter vetch on liver and pancreas weights are summarized in Table 5. In 2-week-old broilers raw bitter vetch diets (15, 30 and 45 percent) resulted in significant increase in pancreas weight in comparison to control ($P<0.05$), but liver weight only in 45 percent SAD had significant difference with control. These results confirm the finding of Ocieo *et al.* (1980a) results that showed the size of pancreas was greater for chickens given 12, 25 and 35% raw *Vicia ervilia* than control. Probably pancreas is target for canavanine and usually pancreas is affected more than other organs (Thomas and Rosenthal, 1987). Trypsin inhibitor is the other factor that maybe contributes in increasing pancreas weight in chicks fed on raw bitter vetch. Trypsin and chymotrypsin inhibitors have been implicated in pancreatic hypertrophy (Liener, 1980). In addition, Grant (1989) reported a lectin-mediated enlargement of pancreas. There were no significant differences in pancreas weight between control and the most of processing methods used in this experiment, that is in coincides with those canavanine content and also indicate heat ranges that selected in these

processing methods were effective for inactivation of trypsin inhibitors. Dhurandhar and chang (1990) showed that cooking navy bean at 94°C for 1.5 hour inactivated greater than 95% of TI and about 99% of its lectin.

Conclusion: It is concluded that (1) feeding of raw bitter vetch to broilers has detrimental effects on performance of broiler chickens and reduced feed intake may be most important part of observed toxic effect. (2) Canavanine was removed by all processing methods that used in this study, especially GSCD, and probably Canavanine is not the only important contributor to the toxicity of bitter vetch for broiler chicken. (3) More improvements in broiler performance in GSCD and SAD treatments suggest that these treatments probably are more effective in removing the other antinutritional factors in this seed and/or they would result in fewer losses in nutrient content of bitter vetch.

Acknowledgements

The authors would like to thanks the Isfahan University of Technology, Isfahan, Iran for financial support during this research. Particular thanks also go to Dr. J. S. Sim and technicians in Chromatography Laboratory at the University of Alberta, Canada for financial and technical assistance in canavanine measurement.

References

Acamovic, T. and J.P.F. D'Mello, 1990. HPLC analysis of canavanine and canaline in canavalia ensiformis, and in excreta and serum of chicks. J. Sci. Food Agri, 50: 63-77.

Aletor, V.A., A.V. Goodchild and Abd El Moneim, 1994. Nutritional and antinutritional charactristic of selected Vicia genotypes. Anim. Feed Sci. Tec., 47: 125-139.

Association of Official Analytical Chemists, 1990. Official methods of analysis (15th ed.) Washington, DC, Association of Official Analytical Chemists.

Austic, R.E., 1986. Biochemical description of nutritional effects. In: Fisher, C., Boorman, K.N. (Eds.), Nutrient Requirements of Poultry and Nutritional Research. Butterworths, London, pp: 59-77.

Barbour, E.K., M. Kallas and M.T. Farran, 2001. Immune response to Newcastle disease virus in broilers: a useful model for the assessment of detoxification of ervil seeds. Rev. Sci. Tech. Off. Int. Epiz., 20: 785-790.

Belmar, R. and T.R. Morris, 1994. Effect of raw and treated jack beans (Canavalia ensiformis) and of canavanine on the short term feed intake of chicks and pigs. J. Agri. Sci., 123: 407-414.

Berger, J.D., L.D. Robertson and P.S. Cocks, 2003. Agricultural potential of Mediterranean grain and forage legumes: 2) Anti-nutritional factor concentrations in the genus Vicia. Genetic Resours and Crop Evolution., 50, 201-212.

Dhurandhar, N.V. and K.C. Chang, 1990. Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cystein content of navy and red kidney beans (Phaseolus vulgaris). J. Food Sci., 55: 470-474.

D'mello, J.P.F., 1995. Anti-nutritional substance in legume seeds. In: D'Mello, J.P.F and Devendra, C. (Eds), Tropical Legumes in Animal Nutrition, CAB International. Walingford, UK., pp: 95-134.

D'mello, J.P.F. and A.G. Walker, 1991.Detoxification of jack beans (Canavalia ensiformis) : studies with young chicks. Anim. Feed Sci. Tec., 33: 117-127.

D'Mello, J.P.F., A.G. Walker and E. Noble, 1990. Effect of dietary supplements on the nutritive value of jack beans (Canavalia ensiformis) for the young chick. Br.Poult. Sci. 31: 759-768.

Ergun, A., S. Yalchin., I. Coplan., S. Yildiz and A.G. Onol, 1990. Utilization of wild vetch seeds (*Vicia ervilia*) by laying hen. Nutr. Abs. Rew., 60: 1008.

Farran, M.T., G.W. Barbour., M.G. Uwayjan and V.M. Ashkarian, 2001a. Metabolizable energy values and amino acid availability of Vetch (Vicia sativa) and Ervil (*Vicia ervilia*) seeds soaked in water and acetic acid. Poult. Sci., 80: 931-936.

Farran, M.T., P.B. Dakessian., A.H. Darwish., M.G. Uwayjan., H.K. Dbouk., F.T. Seliman and V.M. Ashkarian, 2001b. Performance of broilers and production and egg quality parameters of laying hens fed 60% raw and treated common vetch (*Vicia ervilia*) seeds. Poult. Sci., 80: 203-208.

Farran, M.T., M.G. Uwayjan, A.M.A. Miski, F.T. Sleiman, F.A. Adada and V.M. Ashkarian, 1995. Effects of feeding raw and treated common vetch seed (Vicia sativa) on the performance and egg quality parameters of laying hens. Poult. Sci., 74: 1630-1635.

Fernandez-Figares, I., L. Perez, R. Nielo, J.F. Agulera and C. Prieto, 1995. The effect of heat treatment on ileal amino acid digestibility of growing broilers given vetch and bitter vetch meals. Anim. Sci., 63: 493-497.

Fornestedt, N. and J. Porath, 1975. Characterization studies on a new lectin found in seeds of *Vicia ervilia*. FEBS Letters, 57: 187-191.

Geona, M., F. Marzo, A.L. Fernandez-gonzalez, A. Tosar, G. Fruhbeck and S. Santidrian, 1989. Effect of the raw legume *Vicia ervilia* on muscle and liver protein metabolism in growing rats. Rev. Esp. Fisiol., 45: 55-59.

Grant, G., 1989. Anti-nutritional effects of dietary lectins. In: D'Mello, J.P.F., Duffus, C.M., Duffus, J.H.(Eds.), Anti-nutritional Factors, Potentially Toxic Substances in Plants. Association of Applied Biologist Publishing, Wellsbourne, Warwick, pp: 51-74.

Grenson, M., J.M. Mousset and J. Bechet, 1966. Multiplicity of the amino acid permeases *Sacharomyces cerevisiae*. I. Evidence for a spesific arginine-transporting in system. Biochim. Biophys. Acta., 127: 325-338.

Harbak, A., T. Bajor and A. Temesi, 1994. Comparison of substrate and inhibitor specificity of arginase and nitric oxide (NO) synthase for arginine analogues and related compounds in murine and rat macrophages. Biochem. Biophys. Res. Comm., 14: 206-212.

Kadam, S.S., R.R. Smithad, M.D. Eyre and D.G. Armstrong, 1987. Effect of heat treatments of antinutritional factors and quality of proteins in winged bean. J. Sci. Food Agri., 39: 267-275.

Larbier, M., 1987. Digestion and Absorbtion of amino acids. In 6th European .,Sumposium on Poultry Nutrition. World's Poultry Science Association, Konigslutter, pp: RT7.

Lasheras, B., J. Bolufer, M.N. Cenarruzabeitia, M. Lluch and J. Larralde, 1980. Effect of raw legume diets on intestinal absorption of D-galactose by chick. Rev. Esp. Fisiol., 36: 89-92.

Li, E.T.S. and G.H. Anderson, 1983. Amino acids in the regulation of food intake. Nutr. Abs. Rev., 53: 169-181.

Liener, I.E., 1980. Toxic constituents of plant foodstuffs. Academic press, Newyourk, USA, pp: 73-110.

López Bellido, L., 1994. Grain legumes for animal feeds. In : J.E. Hernándo Bermejo and J. León (eds.). Neglected Crops: 1492 from a Different Perspective. Plant Production and Protection Series No. 26. FAO, Rome, Italy, p: 273-288.

Michelagel, C. and R.E. Vargas, 1994. L-canavanine influence feed intake, plasma basic amino acid concentration and kidney arginase activity in chicks. J. Nutr., 124: 1081-1087.

National Research Concil, 1994. Nutrient requirement of poultry, 9th edition. National Academic Press, Washington, D.C.

Ocio, E., R. Vinaras, J. Tervino and A. Brenes, 1980a. Utilization of seeds of *Vicia ervilia* in the feeding of chickens. 1. Effect of the inclusion of high levels of seed for finishing chickens. Avances En Alimentacion-y Mejora Animal, 21: 467-470.

Ocio, E., R. Vinaras, J. Tervino and A. Brenes, 1980b. Utilization of seeds of *Vicia ervilia* in the feeding of chickens. 2. Effect of low levels of seed for starting chickens. Avances En Alimentacion-y Mejora Animal, 21: 525-528.

Ologhobo, A.D., D.F. Apata and A. Oyejide, 1993. Utilisation of raw jackbean (Canavalia ensiformis) and jackbean fractions in diet for broiler chicks. Br. Poult. Sci., 34: 323-337.

Rosenthal, G., 1977. The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. Quart. Rev. Biol., 52: 155-178.

Rueda, E., C. Michelangeli and F. Gonzalez-mujica, 2003. L-canavanine inhibits L-arginine uptake by broiler chicken intestinal bursh border membrane vesicles. Br. Poult. Sci., 44: 620-625.

Santidrian, S., F. Marzo, B. Lasheras, M.N. Cenarruzabeitia and J. Larralde, 1980. Growth rate and composition of muscle of chickens fed different raw legume diets. Growth, 44: 336-342.

SAS Institute, 1995. SAS/STATE User Guide. Version 6.12, SAS Institute Inc, Cary, NC, USA.

Sedgwick, G.W., T.F. Fenton and J.R. Thompson, 1991. Effect of protein precipitating agents on the recovery of plasma free amino acids. Can. J. Anim. Sci., 71: 953-957.

Shqueir, A.A., D.L. Brown and K.C. Klasing, 1989. Canavanine content and toxicity of sesbania leaf meal for growing chicks. Anim. Feed Sci. Tec., 25: 137-147.

Sibbald, I. R., 1979. A bioassay for available amino acid and the true metabolizable energy in feeding stuffs. Poult. Sci., 58: 668-673.

Tepal, J.A., R. Castellanos and A. Larios, 1994. Detoxification of jack beans (Canavalia ensiformis): 1- Extrusion and canavanine elimination. J. Sci. Food Agri., 66: 373-379.

Thomas, D.A. and G.A. Rosenthal, 1987. Toxicity and pharmacokinetics of the nonprotein amino acid L-canavanine in the rat. Toxicol. Appl. Pharmacol., 91: 395-405.

Udedie, A.B.I., 1991. Relative effects of heat and urea treated jackbean (Canavalia ensiformis) on the performance of laying hens. Livestock Research for Rural Development, 3 (3), Online edition, www. cipav. org. co/lrrd/ lrrd3/3/nigeria1.htm