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Serum Proteins and Some Biochemical Parameters in Broiler Chickens Fed with Raw and Treated Bitter Vetch (*Vicia ervilia*) Seeds

[1]Gh. Sadeghi and [2]J. Pourreza
[1]University of Kurdistan, Kurdistan, Iran
[2]Isfahan University of Technology, Isfahan, Iran

Abstract: This study carried out to evaluate the effect of bitter vetch seeds on serum proteins and biochemical parameters in broiler chickens. A total of 1320 one-day old broiler chicks of a commercial breed were placed in 64 pens. Treatments were included raw and four different processed bitter vetch seeds in three levels (150, 300 and 450 g kg^{-1}) and a corn-soybean based diet as control. Each treatment group consisted of four replicates. Processing methods were included soaked in water for 12 h, autoclaved, then dried at room temperature (SAD); ground, soaked in water for 24 h, autoclaved and dried (GSAD); ground, soaked in water for 47 h with exchange water every 12 h, cooked and dried (GSCD) and ground, soaked at 1% acetic acid solution for 24 h at 60°C (AA). Feeding raw, AA and GSAD seeds decreased serum albumin significantly ($p<0.05$) in 21 days old chicks. Chickens that fed with raw and treated bitter vetch seed had lower α_1 and γ globulins than control ($p<0.05$). Increasing raw and treated bitter vetch seeds from 15 to 30 and 45% decreased albumin, α_1 and γ globulins and increased α_2 and β globulins significantly ($p<0.05$). In 14 days old chicks feeding raw and treated biter vetch had no effect on serum urea, but uric acid concentration decreased significantly ($p<0.05$). Feeding SAD seeds increased serum urea significantly ($p<0.05$), but uric acid concentration did not change with feeding raw and treated bitter vetch seeds in 42 day old chicks. Adding raw and treated bitter vetch seeds to diet increased T_4 and decreased T_3 concentrations in all ages. At 28 days old chicks, feeding raw and treated biter vetch seeds decreased alkaline phosphatase concentration significantly than control. Results showed that raw bitter vetch seeds have some toxic effects on metabolism in broiler chickens and GSCD and SAD treatments were more effective to detoxification of this seed.

Key words: Bitter vetch, processing, serum proteins, broiler

INTRODUCTION

Bitter vetch (Vicia ervilia) seeds are produced in the Asian and Mediterranean regions and are a cheap and rich source of energy and protein (Farran *et al*., 2001a). The use of processed bitter vetch seed in poultry diet has been reported (Fernandez-Figares *et al*., 1995; Farran *et al*., 2001b; Sadeghi *et al*., 2004). Use of raw bitter vetch seeds in poultry diet is restricted by the presence of various factors which are toxic for monogastric animal including protease inhibitors, lectins, tannins and canavanine. Canavanin accounts for most toxic effect of bitter vetch seed in monogastric animals.

To improve the nutritional quality and to provide effective utilization of bitter vetch for non ruminant animals it is essential that anti-nutritional factors be removed or reduced. So, it is necessary to establish a processing technique(s) to insure its optimal utilization. In order to inactivate or reduce anti-nutrients, various conventional, simple processing methods have been used in legume seeds (Barbour *et al*., 2001; Farran *et al*., 2001b). Heat treatment is an effective method of inactivating the lectin and protease inhibitors component, however the growth performance of chickens fed with heat treated seeds is still depressed (Farran *et al*., 2001a) and might be attributed to canavanine or other unknown anti-nutritional factors in this seed. So, in the present study we evaluated some complex processing methods to improve nutritional quality of bitter vetch seeds for broiler chickens. The methods used to assess the toxic effects of legume seeds in poultry diets include the determination of one or more productive parameters such as weight gain, feed consumption or feed efficiency. In this study, we determined the serum proteins and biochemical characters of broiler chicken to determine the effective detoxification method of bitter vetch seeds.

MATERIALS AND METHODS

A total of 1320 one-day old broiler chicks of a commercial breed were placed in 64 pens. Feed and water were provided *ad libitum*. The chicks were allocated

Corresponding Author: Gh. Sadeghia, University of Kurdistan, Kurdistan, Iran

randomly to 16 experimental diets. Treatments were included raw and four different processed bitter vetch seeds in three levels (150, 300 and 450 g kg^{-1}) and a corn-soybean based diet as control. Each treatment group consisted of four replicates. Processing methods were included soaked in water for 12 h, autoclaved, then dried at room temperature (SAD); ground, soaked in water for 24 h, autoclaved and dried (GSAD); ground, soaked in water for 47 h with exchange water every 12 h, cooked and dried (GSCD); ground, soaked at 1% acetic acid solution for 24 h at 60°C (AA) bitter vetch seed in three levels (15, 30 and 45%). The diets (Table 1) were formulated to meet nutrient requirements according to NRC (1994).

Blood samples were obtained at 14, 28 and 42 days and the levels of urea (Berthelot method), uric acid (Reece and Hobbi method) were determined by direct colorimetric method using Parsazmun® and Ziestshimi® kits and creatinphosphokinase (CPK), alkaline phosphatase (ALP) and cortisol (Radioimmunoassay method) were determined using commercially available kits (Immunotech company kit). T_3 and T_4 were determined by radioimmunoassay (RIA) method using Kaveshiar® coated tubes. Albumin, α_1 globulin, α_2 globulin, β globulin and γ globulin were measured by gel chromatographyand then albumin to globulins ratio was calculated. 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 Duncan's Multiple Range Test (SAS Institute, 1995).

RESULTS AND DISCUSSION

Serum proteins: Serum proteins at 14 days old broilers fed with raw and treated bitter vetch have been shown in Table 2. Feeding raw, AA and GSAD seeds decreased serum albumin significantly ($p<0.05$). Also, increasing raw and treated bitter vetch seeds from 15 to 30 and 45% decreased albumin significantly ($p<0.05$). Chickens that fed with raw and treated bitter vetch seed had lower α_1 and γ globulins than control ($p<0.05$). Feeding raw bitter vetch seed resulted to a significant ($p<0.05$) increase in α_2 and globulins. Increase in raw and treated bitter vetch seeds from 15 to 30 and 45% decreased α_1 and γ globulins and increased α_2 and β globulins significantly ($p<0.05$). Feeding raw and treated bitter vetch seed, also increase in its level in the diet lead to significant ($p<0.05$) or numerical decrease in albumin to globulins ratio.

The changes in serum albumin, globulins and albumin to globulins ratio in 28 day old chicks fed with different levels of raw and treated bitter vetch seed were similar to 14 days old chicks except for α_1 globulin that dose not detected and for γ globulin that showed no significant difference (Table 3). In 42 day old broilers serum protein changes were similar to 28 day old chicks (Table 4).

Decrease in serum proteins when chicks fed with raw and some treated seeds in comparison to control, could be attributed to presence of toxic level of L-Canavanine in these seeds. Decreased serum proteins is consistent indicators of the hepatotoxicity of canavanine in chickens. L-Canavanine, the guanidinooxy structural

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

	1-21 days				21-42 days				42-49 days			
Ingredient	0	15	30	45	0	15	30	45	0	15	30	45
Corn	573.40	507.20	436.30	366.00	657.80	589.30	420.40	448.90	680.00	611.00	540.90	473.00
Soybean	318.70	242.00	167.70	93.00	264.70	189.20	114.00	39.30	249.60	174.30	99.40	23.40
Bitter vetch	0.00	15.00	30.00	45.00	0.00	15.00	30.00	45.00	0.00	15.00	30.00	45.00
Fish meal	30.00	30.00	30.00	30.00	15.00	15.00	15.00	15.00	0.00	0.00	0.00	0.00
Vegetable fat	40.50	33.00	27.00	21.00	27.60	21.00	14.60	9.90	37.00	30.50	25.00	18.00
Oyster shell	14.50	14.50	15.00	15.10	15.50	15.50	15.10	15.00	16.00	15.90	15.50	15.20
Dicalcium phosphate	13.00	12.50	13.00	13.50	10.60	10.60	11.00	11.50	9.70	10.10	10.50	10.50
D-L Methionin	1.70	1.70	1.80	1.85	0.80	0.80	0.80	0.80	0.30	0.50	0.60	0.80
Lys- HCl	0.00	0.90	1.00	1.50	0.00	0.60	1.10	1.60	0.00	0.50	0.90	1.50
Na Cl	3.20	3.20	3.20	3.20	3.00	3.00	3.00	3.00	2.60	2.60	2.60	2.60
Mineral premix[a]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin premix[b]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Calculated composition												
ME (MJ kg^{-1})	12.73	12.73	12.73	12.73	12.79	12.79	12.79	13.10	13.10	13.10	13.10	13.10
CP (g kg^{-1})	218.20	218.20	218.20	218.20	191.00	191.00	191.00	191.00	176.00	176.00	176.10	176.20
Met (g kg^{-1})	5.20	5.10	5.10	5.10	4.00	3.90	3.90	3.90	3.30	3.30	3.30	3.30
Lys (g kg^{-1})	12.80	12.80	12.80	12.80	10.50	10.50	10.50	10.50	9.30	9.30	9.30	9.30
AP (g kg^{-1})	4.40	4.40	4.30	4.30	3.50	3.50	3.50	3.50	2.90	2.90	2.90	2.90
Ca (g kg^{-1})	9.50	9.50	9.50	9.50	8.70	8.70	8.70	8.70	7.80	7.80	7.80	7.80

[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 raw and treated bitter vetch seeds on serum proteins (g dL^{-1}) at 14 days old broilers

		Globulins				
Treatments	Albumin	α_1	α_2	β	γ	Albumin/ globulins
Control	1.50a	0.100a	0.50b	0.70b	1.25a	0.66a
Raw	1.05c	0.033b	0.68a	0.80a	0.62b	0.51ab
AA	1.07c	0.017bc	0.55b	0.77ab	1.06a	0.49b
GSCD	1.17bc	0.017bc	0.62ab	0.73ab	0.80ab	0.55ab
SAD	1.24bc	0.020bc	0.58b	0.70b	0.76b	0.62ab
GSAD						
Level	1.32b	ND	0.56b	0.72b	0.80ab	0.65a
15%	1.36a	0.044a	0.47b	0.81a	0.75b	0.66a
30%	1.11b	ND	0.67a	0.72b	0.83a	0.53b
45%	1.06b	0.011b	0.65a	0.71b	0.84a	0.49b[a-b]

Values with no common following letter in each column differ significantly ($p<0.05$) ND: Not Detected

Table 3: Effect of raw and treated bitter vetch seeds on serum proteins at 28 days old broilers

		Globulins				
Treatments	Albumin	α_1	α_2	β	γ	Albumin/ globulins
Control	1.80a	ND	0.60b	0.45ab	0.60	1.08a
Raw	1.15bc	0.016	0.70b	0.45ab	0.63	0.64b
AA	1.00c	ND	0.87a	0.48a	0.71	0.51c
GSCD	1.23b	ND	0.72b	0.45ab	0.65	0.68b
SAD	1.08bc	ND	0.63b	0.48a	0.71	0.62b
GSAD						
Level	1.01c	ND	0.83a	0.42ab	0.70	0.52c
15%	1.32a	0.01a	0.71b	0.42b	0.74a	0.72a
30%	1.01b	ND	0.75ab	0.49a	0.69ab	0.53b
45%	0.96b	ND	0.79a	0.46a	0.62b	0.51b[a-c]

Values with no common following letter in each column differ significantly ($p<0.05$) ND: Not Detected

Table 4: Effect of raw and treated bitter vetch seeds on serum proteins at 42 days old broilers

		Globulins				
Treatments	Albumin	α_1	α_2	β	γ	Albumin/ globulins
Control	1.7a	ND	0.50c	0.50ab	0.90a	0.87a
Raw	1.08bc	ND	0.66ab	0.48ab	0.90a	0.53d
AA	1.00c	ND	0.62bc	0.42b	0.88a	0.51d
GSCD	1.28b	ND	0.58c	0.42b	0.76ab	0.71b
SAD	1.12b	ND	0.68ab	0.42b	0.84ab	0.61c
GSAD						
Level	0.86d	ND	0.72a	0.54a	0.70b	0.47e
15%	1.40a	ND	0.60b	0.36c	0.86	0.79a
30%	1.11b	ND	0.64ab	0.43b	0.82	0.59b
45%	0.86c	ND	0.69a	0.53a	0.79	0.44c[a-d]

Values with no common following letter in each column differ significantly ($p<0.05$) ND: Not Detected

analog of L-arginine, can lead to the production of canavanine-containing proteins, which ultimately can disrupt critical reactions of RNA and DNA metabolism and resulting in general inhibition of protein synthesis. 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). 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). Lower serum proteins with higher levels of bitter vetch seeds show the higher toxic substances that interrupt protein metabolism. Higher serum protein fractions in 28 and 42 day old chicks that fed with GSCD seeds shows that this treatment were more effective for inactivation of canavanine and other toxic factor in bitter vetch.

Serum biochemical analysis: Some serum biochemical parameters in broilers fed with raw and treated bitter vetch have shown in Table 5-7. In 14 days old chicks feeding raw and treated biter vetch had no effect on serum urea, but uric acid concentration decreased significantly ($p<0.05$) (Table 5). Serum urea increased significantly ($p<0.05$) in 28 days old chicks with feeding raw and treated bitter vetch seeds (Table 6). Feeding SAD seeds increased serum urea significantly ($p<0.05$), but uric acid concentration did not change with feeding raw and treated bitter vetch seeds in 42 day old chicks (Table 7). In all ages, increase bitter vetch level from 15 to 30 and 45% lead to an increase in urea concentration and a

Table 5: Effect of raw and treated bitter vetch seeds on serum biochemical parameters at 14 days old broilers

Treatments	Urea (mg L^{-1})	Uric acid (mg L^{-1})	T_3 (ng dL^{-1})	T_4 (ng dL^{-1})	CPK (UL^{-1})	Coritso (nM)	APL (mg L^{-1})
Control	19.00	4.20a	2.60a	180.5ab	1320.5ab	8.65a	1988.0a
Raw	19.16	3.68b	1.80ab	159.3b	1504.8a	7.40a	1644.0b
AA	18.16	4.08ab	1.73b	188.5ab	1454.7a	5.75d	2054.0a
GSCD	18.83	3.96ab	1.85ab	160.8b	1124.7b	7.13ab	2043.7a
SAD	19.60	4.38a	2.04a	202.4a	1317.4ab	6.30dc	1468.6b
GSAD							
Level	17.80	4.04ab	1.98ab	172.0ab	1401.4a	6.52bc	1856.4a
15%	17.8b	4.10	2.01a	190.5a	1332.2	6.54	1972.6a
30%	19.6a	3.96	1.92a	159.0b	1381.9	6.58	1866.2a
45%	18.6ab	4.0	1.67b	179.9ab	1365.7	6.79	1628.7b

[a-d]Values with no common following letter in each column differ significantly ($p<0.05$)

Table 6: Effect of raw and treated bitter vetch seeds on serum biochemical parameters at 28 days old broilers

Treatments	Urea (mg L^{-1})	Uric acid (mg L^{-1})	T_3 (ng dL^{-1})	T_4 (ng dL^{-1})	CPK (UL^{-1})	Coritso (nM)	APL (mg L^{-1})
Control	13.00b	5.70a	3.00a	79.0d	1775.0a	2.70b	2851.5a
Raw	16.00a	4.55a	2.10ab	271.0a	1474.8ab	2.72b	2153.5a
AA	14.83a	4.08b	1.96ab	233.5ab	1550.6a	2.93b	1436.3ab
GSCD	15.17a	4.85a	2.13ab	204.8bc	1202.5b	2.73b	1742.0ab
SAD	15.00a	5.18a	1.90b	178.8c	1540.2a	3.71a	1359.5b
GSAD							
Level	15.50a	4.65ab	1.78b	271.2a	1289.8b	2.95b	1337.2b
15%	14.5b	5.36a	2.49a	183.6c	1571.9a	2.71b	1640.8
30%	15.0b	4.13b	1.80b	292.3a	1439.7a	2.81b	1436.1
45%	16.4a	4.50b	1.64c	219.7b	1223.2b	3.51a	1744.7

[a-c]Values with no common following letter in each column differ significantly ($p<0.05$)

Table 7: Effect of raw and treated bitter vetch seeds on serum biochemical parameters at 42 days old broilers

Treatment	Urea (mg L^{-1})	Uric (mg L^{-1})	T_3 (ng dL^{-1})	T_4 (ng dL^{-1})	CPK (UL^{-1})	Coritso (nM)	APL (mg L^{-1})
Control	15.0b	4.10ab	3.00a	56.0c	1083.0d	11.40	2983a
Raw	14.4b	4.10ab	2.34b	329.4a	2261.6ab	12.18	1232c
AA	16.0b	3.48b	2.18b	233.8b	2026.1bc	17.58	2050b
GSCD	16.2b	3.47b	2.40ab	280.7ab	2384.5a	16.25	3103a
SAD	22.8a	4.66a	2.26b	238.4b	1980.0c	15.38	2988a
GSAD							
Level	13.8b	4.14ab	2.02b	272.0ab	2320.8a	133.33	1819b
15%	15.0b	15.0b	3.10a	102.0c	2479.3a	10.70	2616
30%	16.1ab	16.1ab	2.21b	275.6b	2055.4b	8.75	2322
45%	17.9a	17.9a	1.91c	232.2a	2201.2b	12.60	2419

[a-d]Values with no common following letter in each column differ significantly ($p<0.05$)

decrease in uric acid concentration ($p<0.05$). Increase in urea concentration may be a result of higher turn-over rate of canavanine containing proteins. The arginine like structure enables canavanine to incorporate in polypeptide chains, resulting in structurally abberant canavanine containing proteins (Rosenthal, 1977). D'mello and Walker (1991) reported that canavanine containing proteins degrade very readily than those syntheses, subsequently decrease nitrogen retention in broiler chickens.

Adding raw and treated bitter vetch seeds to diet increased T_4 and decreased T_3 concentrations in all ages. Similar pattern were observed when bitter vetch level increased in diet. It could be attributed to effect of anti-nutritional factors in bitter vetch seeds, especially cyanogenic glycosides, on thyroid gland and iodine metabolism.

Feeding AA, SAD and GSAD diets resulted to a significant ($p<0.05$) decrease in cortisol concentration in 14 day old chicks (Table 5). In 28 days old chicks, feeding SAD seeds resulted to higher concentration of cortisol than others and increasing bitter vetch seeds from 15 and 30 to 45%, increased cortisol concentration significantly ($p<0.05$) (Table 6). Increase in cortisol concentration could affect protein metabolism.

CPK concentration was increased significantly in chickens that fed with GSCD and GSAD diets in 28 and 42 days old chicks. Increase in bitter vetch seed level resulted to an increase in CPK concentration in all ages.

Alkaline phosphatase concentration decrease when 14 days old chicks fed with raw and SAD seeds. At 28 days old chicks, feeding raw and treated biter vetch seeds decreased alkaline phosphatase concentration significantly than control. Feeding raw, AA and SA

treatments significantly ($p<0.05$) reduced alkaline phosphatase in 42 day old chicks (Table 7). This reduction may be an effect of canavanine in these seeds. Rosenthal (1977) showed that the biological effect of canavanine include a reduction of protein and glycoprotein synthesis, inhibition of alkaline phosphatase activity and inhibition of RNA synthesis. In addition, have been shown some lectins cause morphological changes in small intestine and reduction in the activities of intestinal and brush border enzymes such as entrokinase and alkaline phosphatase.

It could be concluded from this study that raw bitter vetch seeds has some toxic effects on metabolism in broiler chickens and GSCD and SAD treatments are more effective to detoxification of this seed.

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