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Amino Acid Digestibility and Energy Value of Bitter Vetch (a Cheap Plant Protein) and Effects of Feeding this Plant Protein on Production and Egg Quality Parameters of Leghorn and Native Layer Strain

Research Article

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ABSTRACT

Three experiments were conducted to evaluate the nutritional value of bitter vetch. Experiment 1 was conducted to determine the metabolisable energy content of bitter vetch. Sixteen adult Leghorn roosters were used. Forty grams of bitter vetch were precision-fed individually to 12 roosters. In addition, 4 birds were not fed and served as a control treatment in the measurement of metabolic fecal and endogenous urinary energy output. Experiment 2 was a broiler chick bioassay carried out with 24 male boiler chickens (21-d-old) to determine apparent amino acid digestibilities of bitter vetch. Experiment 3 was conducted to evaluate the effect of bitter vetch on performance and egg quality of two strains of laying hens. A control diet based on corn and soybean meal and diets containing 50, 100 and 150 g kg^{-1} bitter vetch were fed to the Leghorn layers, ISA-Babcock strain, and the native strain. All eggs produced during the last 3 d of each month were collected to evaluate egg quality. True metabolisable energy (TME) and nitrogen-corrected true metabolisable energy (TMEn) values of bitter vetch were 3396 and 3852 kcal/kg DM, respectively. Second experiment showed that the average amino acid digestibility of bitter vetch (12 amino acids) was 66.65%. Feed intake, egg weight, egg production, and egg mass, were higher and feed conversion ratio was lower in the Leghorn than the native strain. Feed intake and egg production were lower and feed conversion ratio was higher by all bitter vetch diets compared with the control diet. Haugh unit and yolk color were lower (P<0.001) in the Leghorn than the native strain and vice versa in egg shell weight (P<0.001). Eggshell thickness and shape index were not affected by strain (P>0.05). It was concluded that 5% of bitter vetch can be used in laying hen diets without any adverse effect.

KEY WORDS amino acid digestibility, bitter vetch, laying hen, metabolisable energy, performance.

INTRODUCTION

With attention to bovine spongiform encephalopathy (BSE or cow mad disease), using of meat and bone meals derived from cattle and pig are prohibited. Therefore, plant protein has to be used more frequently. Soybean meal is usually known as a suitable source of protein in the poultry diet, but its availability is also low in the Asia. Therefore, in semi-arid regions, another source of protein such as other legume seeds is suggested for substitution in poultry diet (Farran *et al.* 1995). Legumes such as vetch (*Vicia sativa*) and bitter vetch (*Vicia ervilia*) are cultivated extensively in arid regions of central west Asia and north Africa as high-protein forage for animals with increased grain yield and ability to sustain soil fertility (ICARDA, 2004). Despite high levels of protein (200 to 450 g kg^{-1}) and carbohydrate in legume seeds, due to imbalanced amino acid profiles and anti-nutritional factors, these products are poorly used in poultry diets (Marzo *et al.* 2002).

Bitter vetch is a good and inexpensive source of protein and energy (Farran *et al.* 2001b; Mikic *et al.* 2006; Sadeghi *et al.* 2008). The legume is frequently used as a source of protein in poultry diet (Farran *et al.* 2001b; Fernandez-Figares *et al.* 1993). Broiler diets supplemented with 150, 250 and 350 g kg-[1] bitter vetch had no effect on mortality but decreased weight gain and feed conversion ratio when fed to birds over a period of 4 wk (Ocio *et al.* 1980). It is also reported that addition of 40-120 g kg^{-1} bitter vetch to the layer rations for 6 months, negatively affected live weight, feed conversion ratio, egg production, and egg weight (Ergun *et al.* 1993).

There is a paucity of information on metabolisable energy and digestibility of amino acids in bitter vetch for poultry. Data regarding the effects of bitter vetch on egg quality parameters in laying hens are also sparse. Therefore, the objective of this study was determination of the ME and apparent amino acid digestibilities of bitter vetch. In addition, performance and egg characteristics of two strains of hens (native and Leghorn) in response to bitter vetch diets were considered.

MATERIALS AND METHODS

This study was carried out in animal research station of Bu Ali Sina University at March 2012 in Hamedan Iran.

Experiment 1

Proximate analysis and metabolisable energy

For proximate analysis, all samples were dried in a forced air oven at 60 ˚C for 48 h and ground to pass through 1 mm screen. Moisture, crude protein, ether extract, crud fiber and ash content of samples were determined by AOAC Methods (AOAC, 1990). True metabolisable energy (TME) and nitrogen corrected true metabolisable energy (TMEn) of the test ingredient were determined by Sibbald method (1986).

Experiment 2

Amino acid profile, digestibility and modified limiting amino acid score

Amino acid digestibilities were determined according to the method of Newkirk *et al.* (2003) using 24 male boiler chickens raised to 21 d of age. Then, birds were randomly placed in the colony cages at two birds per cage. The cages (experimental units) were randomly assigned to one of two dietary treatments (Table 1). The diets were fed to the birds for 7 d.

Chromic oxide was included in all diets as an indigestible marker. Excreta were collected daily for the last 3 d and frozen upon collection. Amino acids in the diets and excreta samples were measured by Siriwan *et al.* (1993) method.

Table 1 Composition and calculated nutrient contents of diets used in the amino acid digestibility experiment (g kg^{-1})

Ingredient	Basal diet	Test diet
Corn grain	671.5	345.8
Soybean meal (44%)	242.5	188.2
Bitter vetch	-	400.0
Vegetable oil	30.0	10.0
Fish meal	7.5	7.5
Oyster shell	18.5	18.5
Calcium H phosphate[a]	16.0	16.0
Salt	4.0	4.0
Chromium (III) oxide	5.0	5.0
Vitamin premix[b]	2.5	2.5
Mineral premix[c]	2.5	2.5
Total	1000	1000
Calculated nutrient contents (as-fed basis)		
ME (kcal/kg)	3083.00	2870.82
Protein	161.5	196.0
Lysine	9.5	13.0
Met + Cys	6.3	11.0
Ca	11.3	15.2
Available P	4.3	5.9

[a] Contains 210 Ca and 180 available P (g kg-1).
[b] Vitaminl premix supplied per kg of diet: Thiamin HCl: 3.3 g; Riboflavin: 0.72g; Menadione dimethylpyrimidinol bisulfate: 1.6 g; DL-α-tocopherol acetate: 14.4 g; Cholecalciferol: 7 g; Retinyl acetate: 7.7 g; D-Ca-pantothenate acid: 12 g; Pyridoxine HCl: 6.2 mg; B_{12}: 14.4 g and Choline: 440 mg.
[c] Mineral premix supplied per kg of diet: $MnSO_4$ H_2O: 64 g; $ZnCO_3$: 44 g; $FeSO_4$ $7H_2O$: 100 g; $CuSO_4$ $5H_2O$: 16 g and KI: 0.64 g.

Briefly, defatted samples were hydrolyzed under nitrogen with 6 N hydrochloric acid containing phenol (3 g/L) at 120 ˚C for 24 h.

These samples were adjusted to pH 2.3 prior to separation by ion-exchange and detection by high-performance liquid chromatography (HPLC).

Chromium **(III)** oxide was measured according to the method of Fenton and Fenton (1979). Amino acid digestibility in diets was calculated by the following formula (Newkirk *et al.* 2003):

$$Dc_{diet} = 1 - [(M_{diet}/M_e) \times (AAC_e/AAC_{diet})]$$

Where:
DC_{diet}: digestibility coefficient of amino acid in diet.
M_{diet}: marker concentration in diet.
M_e: marker concentration in excreta (e).
AAC_{diet}: concentration of amino acid in diet.
AAC_e: concentration of amino acid in excreta (e).

Amino acid digestibility in bitter vetch was calculated by the following formula (Newkirk *et al.* 2003):

$$DC_{bitter\ vetch} = [((DC_{test} - DC_{basal} \times 0.6)/(4)) \times 10]$$

Where:
$DC_{bitter\ vetch}$: digestibility coefficient of amino acid in the bitter vetch.
DC_{test}: digestibility coefficient of amino acid in the test diet. DC_{basal}: digestibility coefficient of amino acid in the basal diet.

Modified limiting amino acid score (MLAAS) of bitter vetch seeds for broilers (0-3 week) and laying hens was calculated on the basis of following formula (Sadeghi *et al.* 2008).

MLAAS= (amino acid percent of test protein/requierd amino acid percent of requierd protein for test animal)

Experiment 3
Layer performance
To evaluate the effect of bitter vetch on performance and egg quality of two strains of laying hens, one hundred fifty-four Single Comb White Leghorn layers of ISA-Babcock strain and 154 native strains (both aged 13 wk) were used. The growing diet was formulated to meet NRC (1994). Pullets were given 12 h of light during the grower period. At 18 wk of age, all birds were transferred to the production house and each 3 hens were housed in a conventional type cage (42×40×50 cm^3). The temperature of housing was in the range of 18 to 22 ˚C; relative air humidity ranged from 65 to 70%.

A control diet was provided *ad libitum* for a 4-wk depletion period. The remaining birds, at the end of the pre-experimental feeding stage, were weighed and assigned to the 4 experimental diets (containing 0, 50, 100 and 150 g kg^{-1} of raw bitter vetch) for 6 months in a 2 × 4 factorial arrangement. Four replicates of 9 hens were fed from each diet. The experimental diets (Table 2) were formulated to meet NRC nutrient requirements for layers.

The experimental period was divided into 6 subperiods of 1 month each. At the end of each subperiod, feed intake, egg weight, egg production, egg mass and feed conversion ratio were recorded. All the eggs produced during the last 3 d of each subperiod were collected and weighed. Egg size index, shell thickness and shell weight, Haugh unit score, yolk color and yolk index were measured. At the end of the experiment, body weight and mortality were also recorded for each treatment. Experiments 1 and 2 were set up as completely randomized designs. Data were analyzed by one-way analysis of variance (ANOVA) using the general liner model (GLM) procedure of SAS software (SAS, 2004).

Table 2 Composition and calculated nutrient contents of diets used in laying period (%)

Ingredient	Control	(Diet 1)	(Diets 2)	(Diets 3)
	Diets			
Corn	62.35	59.44	56.36	53.28
Soybean meal (44%)	26.50	24.51	22.57	20.98
Bitter vetch	-	5.00	10.00	15.00
Soybean oil	0.56	0.54	0.54	0.54
Limestone	8.11	8.10	8.15	8.19
Dicalcium phosphate[a]	1.55	1.54	1.32	1.10
Common salt	0.39	0.33	0.35	0.37
Vitamin permix[b]	0.25	0.25	0.25	0.25
Mineral permix[c]	0.25	0.25	0.25	0.25
DL-Met (98.5%)	0.04	0.04	0.04	0.04
Total	100	100	100	100
Calculated nutrients (as-fed)				
ME (kcal/kg)	2800	2800	2800	2800
Crude protein	17.00	17.00	17.05	17.00
Ca	3.44	3.48	3.49	3.50
Available p	0.44	0.45	0.43	0.40
Na	0.17	0.16	0.16	0.17
Met	0.34	0.34	0.34	0.34
Lys	0.97	0.98	0.99	0.10
Met + Cys	0.64	0.64	0.65	0.64

[a] Contains 187 P and 220 Ca (g kg-1).
[b] Vitaminl premix supplied per kg of diet: Thiamin HCl: 3.3 g; Riboflavin: 0.72g; Menadione dimethylpyrimidinol bisulfate: 1.6 g; DL-α-tocopherol acetate: 14.4 g; Cholecalciferol: 7 g; Retinyl acetate: 7.7 g; D-Ca-pantothenate acid: 12 g; Pyridoxine HCl: 6.2 mg; B_{12}: 14.4 g and Choline: 440 mg.
[c] Mineral premix supplied per kg of diet: $MnSO_4$ H_2O: 64 g; $ZnCO_3$: 44 g; $FeSO_4$ $7H_2O$: 100 g; $CuSO_4$ $5H_2O$: 16 g and KI: 0.64 g.

In experiment 3, a two-way analysis of variance was used to assess the main effects of strain (S) and diet (D) and the corresponding interaction S × D.

RESULTS AND DISCUSSION

Experiment 1
Proximate analysis and metabolisable energy
Dry matter, crude protein, ether extract, crude fiber and ash contents of bitter vetch were 944, 216.5, 17.9, 48.2 and 37.4 g kg^{-1}, respectively. The TME and TMEn of bitter vetch were 3396 and 3852, respectively.

Experiment 2
Amino acid profile, digestibility and modified limiting amino acid score
In this experiment, the apparent amino acid digestibilities of bitter vetch ranged from 56.55 to 75.89% with a mean value of 66.65%. In broilers, the mean values of MlAAS in soybean meal and bitter vetch were 136.14 and 115.92%, respectively.

The MlAAS values for soybean meal and bitter vetch in laying hens were 188.79 and 151.59%, respectively (Table 3).

Experiment 3
Laying performance
Feed intake of control diet in native hens was significantly lower (P<0.001) than the control diet in leghorn, while the effect of strain on feed intake was not significant for the other corresponding dietary treatments (P>0.001). Except for the native hens fed diet containing 50 g kg^{-1} of bitter vetch, compared to the control diet, feed intake was significantly (P<0.001) reduced with increasing bitter vetch levels in the diets (Table 4). Egg weight produced by hens fed control diet was significantly higher in the Leghorn than the native hens (56.08 *vs*. 53.10%, P<0.001). Egg weight in both strains was reduced with increasing bitter vetch from 50 to 150 g kg^{-1} in the diets. The lowest egg weight was consistently produced by 150 g kg^{-1} bitter vetch in the diets. The highest egg production was obtained with the leghorn hens fed the control diet (78.20%). For diet by strain interaction, increasing the level of bitter vetch in the diets decreased (P<0.05) average egg production and egg mass in both stains (Table 4). The feed conversion ratio (FCR) was inferior in the native strain than the leghorn strain for all dietary treatments. In both strains, FCR was continually improved with decreasing bitter vetch level in the diets.

Egg quality
Egg shell weight was numerically higher in the Leghorn strain than the native strain (4.73 *vs*. 4.69 g). The lowest eggshell weigh was observed in 100 and 150 g kg^{-1} bitter vetch diets compared to the control diet (P<0.01). No significant interactions were observed between strain and diet for egg shell weight and eggshell thickness (P>0.05). Egg quality as assessed by Haugh unit and yolk color was significantly higher in the native strain than the leghorn (P<0.001), but not affected by the diets (P>0.05). A linear decreasing trend (P<0.001) was observed in yolk index for strain and diet interaction. The shape index of eggs declined with increasing dietary bitter vetch level within each strain (Table 5).

Experiment 1
Proximate analysis and metabolisable energy
The crude protein of bitter vetch in this study (216.5 g kg^{-1}) is nearly close to reported values by Tabatabaei *et al*. (2000), Bakoglu *et al*. (2009) and Abdullah *et al*. (2010), but that is relatively lower than other studies (Farran *et al*. 2001a; Sadeghi *et al*. 2008; Alipour Filabadi *et al*. 2014). The ether extract (17.9 g kg^{-1}) of bitter vetch in the present study is lower than those reported by Tabatabaei *et al*. (2000) and Reisi *et al*. (2011) but is higher than the other studies (Farran *et al*. 2001a; Sadeghi *et al*. 2008). The differences between proximate analyses of this study with those reported in the literature could be related to differences in variety and growth conditions of analyzed seeds.

The increase in TME of bitter vetch as a result of nitrogen correction is in agreement with findings of Farran *et al*. (2001a) and Sadeghi *et al*. (2008) who concluded that bitter vetch could be highly detrimental to the metabolism of nitrogen. The content TMEn of bitter vetch in the present study is fairly consistent with Sadeghi *et al*. (2008) results, but is higher than Farran *et al*. (2001a) report. The metabolisable energy of ingredients is affected by several factors including, fiber, protein, seed quality, anti-nutritional factors and variety (Bell, 1993). Therefore, these factors may be in part responsible for variations in metabolisable energy value of bitter vetch reported in literature.

Experiment 2
Amino acid profile, digestibility and modified limiting amino acid score
Amino acid profile of bitter vetch in this study is nearly similar to those reported previously (Prieto *et al*. 1994; Sadeghi *et al*. 2008) (Table 3). Research on the amino acid digestibilities of bitter vetch in poultry diets is relatively scarce. In the present study, the apparent amino acid digestibility of bitter vetch (*Vicia ervilia*) in comparison to the apparent amino acid digestibility of vetch (*Vicia ervilia*) reported by Farran *et al*. (2001a) was fairly similar for His, Ile, Tyr and Val, while it was lower and higher for Ala, Arg, Asp, Ser and Leu, Lys, Met and Phe, respectively.

Modified limiting amino acid score could be used as a reasonable test for determination protein quality of most grain legumes (Wiryawan and Dingle, 1995). In this study, mean value of MLAAS of BV in broilers was equivalent to 0.85 of MLAAS in soybean meal. In other words, it was concluded that each unit of BV protein was equivalent to 0.85 of soybean meal.

The MLAAS decreased by 5% when it was calculated for laying hen diets. Finally, in spite of the fact that MLAAS is a simplest and quickest test in comparison to the others, this result should be interpreted with caution, because MLAAS is an *in vitro* method in evaluating the protein quality in grain legumes.

Experiment 3
Laying performance
The result of this experiment for feed intake is in agreement with Ocio *et al*. (1980), Ergun *et al*. (1993), Sadeghi *et al*. (2004) and Farran *et al*. (2005) results that showed that consumption of bitter vetch caused lower feed intake in broiler chickens and laying hens.

Table 3 Amino acid profile, apparent amino acid digestibility and modified limiting amino acid scores (MLAAS) of bitter vetch (BV) and soybean meal (SBM)

Amino acids	BV profile[a]	Digestibility[a]	Layer MLAAS[a]	Broiler MLAAS[a]	Mean[b]	SBM profile[c]	Layer MLAAS[c]	Broiler MLAAS[c]
Arg	9.44	75.89	276.71	173.69	0.91±7.59	7.14	208.93	131.37
Gly + ser	12.46	ND[d]	ND[d]	229.26	0.63±4.86	3.46	ND[d]	175.16
His	2.10	71.00	255.02	137.99	0.25±2.45	2.66	322.52	174.79
Ile	3.03	59.39	95.39	87.12	0.02±3.51	4.45	140.08	127.93
Leu	4.11	69.41	102.76	78.77	1.00±5.82	7.70	192.39	147.58
Lys	3.01	56.55	88.23	62.93	1.24±5.71	6.16	178.98	127.75
Met	0.7	67.57	47.60	32.20	0.12±0.92	1.41	95.71	64.86
Phe	4.09	72.05	178.29	130.65	0.43±4.71	4.91	213.74	156.84
Phe + Tyr	6.49	ND	159.91	111.39	0.23±7.25	9.25	227.64	158.76
Thr	4.42	ND	192.68	127.07	0.21±3.34	3.91	170.20	112.41
Val	4.07	68.09	119.35	104.01	0.84±4.65	4.70	137.73	120.11
Ala	4.6	60.51	ND	ND[d]	0.14±4.44	-	ND	ND
Asp	10.11	67.14	ND	ND	1.92±11.44	-	ND	ND
Ser	7	68.18	ND	ND	0.26±5.30	5.2	ND	ND
Glu	16.03	ND	ND	ND	1.00±18.01	-	ND	ND
Gly	5.46	ND	ND	ND	0.16±4.41	4.32	ND	ND
Tyr	2.4	64.02	ND	ND	0.10±2.27	4.34	ND	ND

[a] Amino acid profile, digestibility and MLAAS of bitter vetch (this study).
[b] Mean of amino acid profiles of bitter vetch reported by previous studies (Farran *et al*. 2001a; Sadeghi *et al*. 2008; Prieto *et al*. 1994).
[c] Amino acid profile and MLAAS of soybean meal (NRC, 1994).
ND: not determined.

Table 4 Effect of bitter vetch and strain on laying hen performance from 19 to 43 wks of age

Factor		FI (g/h/d)	EW (g)	EM (g/h/d)	EP (%)	FCR (g/g)
Strain (S)	Leghorn	77.47[a]	55.10[a]	32.61[a]	58.72[a]	2.48[b]
	Native	71.94[b]	52.77[b]	25.76[b]	47.88[b]	2.99[a]
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001
Diet (D)	Control	85.63[a]	54.52[a]	39.51[a]	72.10[a]	2.19[c]
	1 (5% BV)	80.30[b]	54.50[a]	33.79[b]	60.38[b]	2.44[c]
	2 (10% BV)	74.40[c]	53.53[b]	25.63[c]	47.10[c]	2.95[b]
	3 (15% BV)	61.35[d]	52.69[ab]	18.12[d]	33.62[d]	3.36[a]
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001
S × D						
Leghorn	Control	90.77[a]	56.08[ab]	43.97[a]	78.20[a]	2.07[d]
	1 (5% BV)	81.25[b]	55.13[bc]	37.48a[b]	67.61[b]	2.18[cd]
	2 (10% BV)	75.39[cd]	54.20[cd]	28.73c[d]	52.49[c]	2.65[c]
	3 (15% BV)	62.45[e]	53.72[d]	21.04[de]	36.58[de]	3.01[b]
Native	Control	80.48[bc]	53.10[d]	34.71[bc]	66.00[b]	2.31[cd]
	1 (5% BV)	76.9[bc]	53.09[d]	28.60[cd]	53.15[c]	2.7[c]
	2 (10% BV)	71.59[d]	52.81[de]	22.55[d]	41.71[d]	3.25[ab]
	3 (15% BV)	58.77[e]	51.67[e]	15.49[e]	30.66[e]	3.71[a]
	P-value	0.0001	0.0003	0.0212	0.0119	0.0049
	Pooled SEM	1.31	0.28	0.64	1.86	0.23

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
FI: feed intake; EW: egg weight; EM: egg mass; EP: egg production; FCR: feed conversation ratio.
SEM: standard error of the means.

The effects of anti-nutritional factors of legume seeds on feed intake are well established. Therefore, the lower feed intake with consumption of bitter vetch may be related to its anti-nutritional factor, especially canavanine, which is well documented in several studies (Sadeghi *et al.* 2009). The lower feed intake caused by bitter vetch or canavanine in poultry diets is relatively similar (Michelangeli and Vargas, 1994). In contrast, no adverse effect is reported on poultry feed intake with 0.056 g kg^{-1} canavanine for 2 weeks. In fact, plasma normal amino acid profile is severely disrupted with bitter vetch inclusion in the diets. In other words, inclusion of bitter vetch in poultry diets will cause a significant decrease in concentration of plasma histidine compared with the control diet (Michelangeli and Vargas, 1994). Also, feed intake is rapidly reduced by disrupted amino acid pattern of plasma in birds fed excessive or misappropriate amount of amino acids (Austic, 1986). Another possibility for the effect of canavanine may be associated with its inhibition effect on lysine. Canavanine structure is similar to arginine (Rosenthal, 1977). Therefore, D'Mello *et al.* (1990) suggested a canavanine-arginine interaction which parallels with the well-established lysine-arginine antagonism that depresses feed intake. Consistent with the result of this study, a reduction in egg weight with bitter vetch at levels of 120 and 300 g kg^{-1} was reported by Ergun *et al.* (1993) and Farran *et al.* (2005), respectively. Decrease in egg weight was also observed by Farran *et al.* (2005) using (*Vicia sativa*) at levels of 75 and 225 g kg^{-1} in laying hen diet. In the current study, in spite of similarity in age and feed intake between two strains fed on the same dietary treatments, the lower egg weight in the native strain may be related to strain genotype which is one of the most important factors influencing not only egg weight but also other egg characteristics (Zita *et al.* 2009).

The poorer egg production and egg mass found upon feeding of dietary levels of bitter vetch seeds are attributed to feed intake reduction (Mohammadi and Sadeghi, 2009), which is comparable to the findings from this work. The egg production of both strains are confirmed by the results of Ergun *et al.* (1993) and Farran *et al.* (2005), who observed that egg production was reduced with 40 to 120 g kg^{-1} inclusion of bitter vetch in the diets. In addition, a 73.84 to 92.19% decrease in egg production with 300 to 600 g kg^{-1} bitter vetch in the diets was also reported by Farran *et al.* (2005). The inferior FCR and reduced digestion and absorption of nutrients caused by anti-nutritional factor of bitter vetch seeds in birds are documented in several studies (Ergun *et al.* 1993; Farran *et al.* 2005). Besides the lower feed intake of the bitter vetch diets, lower amino acid digestibilities of bitter vetch compared with soybean meal (66.65 *vs.* 89.47%, Saki *et al.* 2008) may be in part responsible for the poorer performance in laying hens. With attention to the results of this study on FCR, inclusion level of bitter vetch up to 50 g kg^{-1} is suggested in laying hen diets.

Egg quality

A paucity of data can be found regarding bitter vetch effect on egg quality in the literature. It is reported that albumen, yolk and shell weight of eggs tend to increase with an increase in egg weight (Chung and Stadelman, 1965). In this work, there was a linear relationship between egg shell weight and dietary bitter vetch levels.

Table 5 Effect of bitter vetch and strain on egg quality of laying hens from 19 to 43 wks of age

Factor		ESW (g)	EST (mm)	HU (%)	YC	SI (%)	YI (%)
Strain (S)	Leghorn	4.73^a	0.37	77.56^b	8.11^b	75.77	0.52^b
	Native	4.69^b	0.37	81.33^a	8.72^a	76.12	1.04^a
	P-value	0.0001	0.4218	0.0001	0.0001	0.0566	0.0001
Diet (D)	Control	4.80^a	0.37	80.87	8.28	76.64^a	1.50^a
	1 (5% BV)	4.75^{ab}	0.37	80.84	8.39	75.98^b	0.75^b
	2 (10% BV)	4.66^b	0.37	80.84	8.32	75.27^c	0.50^c
	3 (15% BV)	4.63^b	0.37	81.33	8.69	75.84^{bc}	0.38^d
	P-value	0.0054	0.0582	0.1868	0.1517	0.0001	0.0001
S × D	P						
Leghorn	Control	4.85	0.37	77.48	7.75	76.81^a	1.00^b
	1 (5% BV)	4.78	0.37	77.44	8.23	75.98^{ab}	0.50^d
	2 (10% BV)	4.68	0.37	77.46	8.21	75.17^{bc}	0.33^e
	3 (15% BV)	4.62	0.37	77.94	8.27	74.99^c	0.25^f
Native	Control	4.75	0.38	84.31	8.81	76.48^a	2.00^a
	1 (5% BV)	4.70	0.37	84.41	8.54	75.98^{ab}	1.00^b
	2 (10% BV)	4.68	0.36	84.50	8.43	75.36^{bc}	0.67^c
	3 (15% BV)	4.61	0.38	84.91	9.10	75.23^c	0.50^d
	P-value	0.1337	0.4415	0.9833	0.0918	0.0046	0.0001
	Pooled SEM	0.24	0.0014	1.31	0.19	12.36	0.20

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
FI: feed intake; EW: egg weight; EM: egg mass; EP: egg production; FCR: feed conversation ratio.
SEM: standard error of the means.

This trend was also observed for egg weight within each strain. Based on the results of Farran *et al*. (2005), treating bitter vetch with soaking in water or acetic acid resulted in a lower egg shell thickness than the control, but there was no effect for untreated bitter vetch on egg shell thickness. Ergun *et al*. (1993) reported that untreated bitter vetch at a level of 120 g kg^{-1} had no effect on egg shell thickness and Haugh unit which is parallel with our results. The higher Haugh unit in the native strain relative to the leghorn strain may be related to genotype differences. In the present study, yolk color was not affected by the bitter vetch diets. This result is consistent with the findings of Ergun *et al*. (1993) and Farran *et al*. (2005) who showed that 100 and 250 g kg^{-1} of bitter vetch had no effect on yolk color. It is suggested that bitter vetch may contain yolk coloring pigments that is not present in untreated bitter vetch (Ergun *et al*. 1993; Farran *et al*. 2005).

CONCLUSION

Results indicated that bitter vetch is an energy and protein rich ingredient. It was concluded that no detrimental effects were found on laying hen performance by 50 g kg^{-1} of bitter vetch in diet. Higher consumption of bitter vetch depressed feed intake, which was accompanied by a proportional reduction in performance. Mean of laying hen MLAAS values of bitter vetch showed that each unit of bitter vetch protein was equivalent to 0.80 of soybean meal protein abilities to provide amino acid requirements in laying hen diets. In finally, further investigation need to be modulated by higher levels and processing aspect in regarding of molecular reaction of bitter vetch.

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